Feasibility of phytoremediation for common soil and groundwater pollutants

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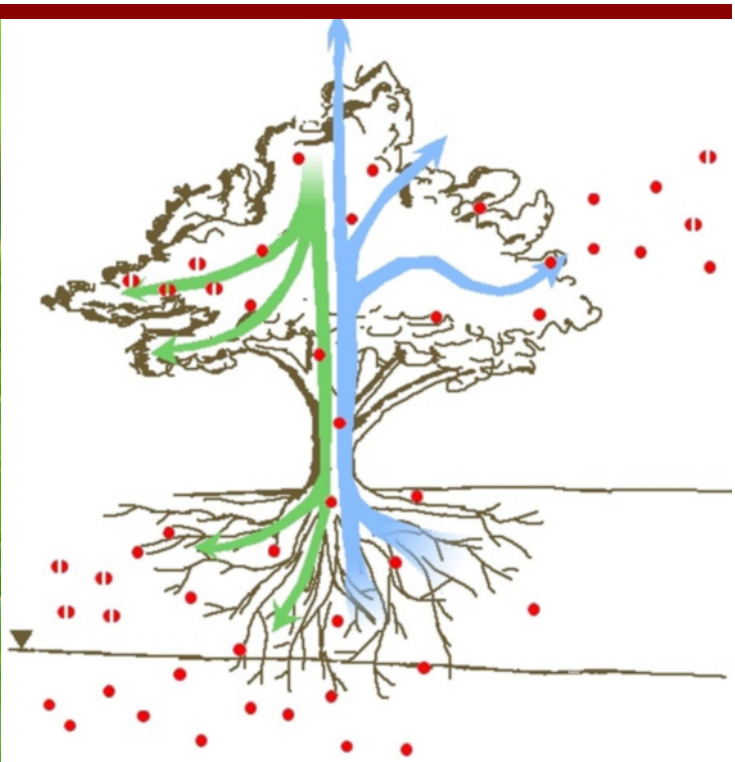

Lauge Clausen

PhD Thesis
October 2017

DTU Environment
Department of Environmental Engineering

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Preface

The research contained in this PhD thesis elucidates the field of phytoremediation and the fate of contaminants when taken up by plants. Special focus is given to the assessment of feasibility and applicability of phytoremediation and to highlight the need for a common testing strategy. The research undertaken was conducted in the period from April 2013 to October 2017 under supervision of Professor Stefan Trapp and co-supervision of Associate Professor Mette Martina Broholm.

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IV**.

Concerning **paper I** it should be noted that the data presented in the paper was generated within the Master project "Toxicity and uptake of fluoride to willows - Recommendations for a contaminated site" by **Clausen LPW**. Further data interpretation, method validation and paper writing was carried out as part of the PhD study.

I **Clausen LPW**, Karlson UG, Trapp S (2015). Phytotoxicity of sodium fluoride and uptake of fluoride in willow trees, *International journal of phytoremediation*, 17(4), 369-376.

II **Clausen LPW**, Broholm MM, Karlson UG, Trapp S (2017). Test of aerobic TCE degradation by willows (*Salix viminalis*) and willows inoculated with TCE-cometabolizing strains of *Burkholderia cepacia*, *Environmental Science and Pollution Research*, DOI 10.1007/s11356-017-9420-8.

III **Clausen LPW**, Trapp S (2017). Toxicity of 56 substances to trees, *Environmental Science and Pollution Research*, DOI 10.1007/s11356-017-9398-2.

IV **Clausen LPW**, Jensen CK, Trapp S (2017). Toxicity of 2,3,5,6-tetrachlorophenol to willows. *Human and Ecological Risk Assessment: An International Journal*. Short communication. *Submitted*.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study:

Trapp S, Rein A, **Clausen LPW**, Algreen M (2014). Feasibility of phytoremediation of common soil and groundwater pollutants. TIMBRE project, FP7- ENV-2010.3.1.5-2, contract no: 265364. Available at: http://www.timbre-project.eu/

Martac E, Trapp S, **Clausen LPW**, Algreen M, Stalder M, Krupanek J, Kalisz M, Fatin-Rouge N (2014). Comparative study of DP-based site investigation approaches and potential in situ remediation techniques: model-assisted evaluation of advantages and uncertainties. TIMBRE project, FP7- ENV-2010.3.1.5-2, contract no: 265364. Available at: http://www.timbre-project.eu/

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I would also like to thank my friends Martin, Søren, Daniel, Eddie, Tobias and Sigurd for providing a weekly safe haven where I could clear my mind and recharge anew.

Lastly, but not least, I would like to thank my family; my mother, father and sister for encouraging me throughout the long journey, my lovely wife for being you and for carrying all the duties at home – I love you, and my two amazing boys for making me laugh and for coping with their father's, from time to time, long absence.

Summary

During the past two to three decades numerous studies reporting highly efficient remediation of contaminated soil and groundwater by plants have been published. The promises of phytoremediation has been great but till now the technology has not been widely applied and recognized, commercially and in a regulatory context, on par with other conventional soil and groundwater remediation technologies.

This thesis elucidates the field of phytoremediation and addresses the lack of recognition of the technology. It aims to assesses the overall feasibility of phytoremediation and identify obstacles within the field. Further, it provides examples and suggestions of how to overcome these obstacles.

The first part of the thesis scrutinizes the literature for data and experiences regarding application of phytoremediation and uncovers potential barriers and where the existing knowledge is insufficient. Further, it considers phytoremediation from a more technical perspective, setting up a mass balance for a generic plant-soil system. On basis of the data review and the mass balance application, an initial assessment of the feasibility of phytoremediation for common soil and groundwater pollutants is conducted.

Several knowledge gaps and limitations were identified. Phytoremediation is restricted by phytotoxicity, root depth and long remediation time. Further, there is an evident lack of information about fate of contaminants in plants and the plant processes affecting uptake, excretion and metabolism of contaminants. These data are needed for mass balance modelling purposes.

The mass balance revealed that if contaminants are degraded (k_{deg} ~ 0.01/day or higher) the governing loss of contaminant mass in the soil matrix is by aerobic microbial degradation in the rhizosphere and not by plant uptake. If the total removal of contaminant mass by degradation, direct volatilization and other unknown processes (not including plant uptake and leaching) is negligible, the governing removal process is plant uptake. However, removal by plant uptake is slow compared to rhizo-degradation and it is controlled by the soil matrix volume and the soil/water distribution coefficient (K_d). For compounds with high K_d (e.g. lead) phytoremediation may take immensely long time (>80,000 years). Extraction efficiency of plants is proportional to the soil volume which explains why pot experiments tend to under estimate

the remediation time. The feasibility of phytoremediation for organic compounds with limited aerobic degradation (e.g. many chlorinated solvents) and low K_d (~10 L/kg) is more difficult to assess due to lack of knowledge of their fate.

The second part of the thesis proposes two flow charts for assessment of the applicability of phytoremediation and applicability of phytoremediation techniques, respectively. They can be seen as a check list of things to consider before implementation of phytoremediation and are meant to ease the decision-support process. Further, they emphasize the need for phytotoxicity data, fate, metabolism- and mass balance studies.

It is shown that existing phytotoxicity tests with higher terrestrial plants, not including seed germination, lack standardization and reporting requirements for easy comparison and transparency. The willow tree acute toxicity test by Trapp et al. (2000) is suggested as a candidate for a standardized test as it allows combined uptake, metabolism and toxicity studies. An overview of phytotoxicity data generated by the willow tree acute toxicity test is provided and compared to results generated by the standardized phytotoxicity tests on freshwater green algae and duckweed. No species could be classified as the most sensitive. It is proposed to establish a phytotoxicity database for standardized tests on terrestrial plants, not including seed germination.

The fate and metabolism of trichloroethylene (TCE) in plants were evaluated by the willow tree acute toxicity test with chloride as indicator of dehalogenation. Willows and willows inoculated with TCE co-metabolizing strains of the plant endophyte *Burkholderia cepacia* were continuously exposed to TCE during a tree week period. Approximately 96% of the added TCE evaporated from solution and only 4% was taken up by the plants. Less than 3% of the added TCE was mineralized.

To assess the feasibility of phytoremediation at a sodium fluoride contaminated site, the uptake of fluoride in plants were determined by the willow tree acute toxicity test. The mass balance revealed that the willows were capable of enzymatic removal of fluoride from the roots at low external concentrations. At high external concentrations the enzyme system collapsed and fluoride was taken up leading to further toxic effects. Phytoremediation was not assessed to be feasible at the site.

The work encompassed in this thesis underlines that phytoremediation may occasionally be feasible. However, there are several limitations which strongly inhibit the applicability. Phytoremediation is assessed to be feasible for nutrients and organic pollutants which can be degraded aerobically. Most trace elements are too strongly bound to the soil matrix to be available for plant extraction and are thus not feasible for phytoremediation. For phytoremediation to become an accepted and recognized technology it must be proven by providing examples of field-scale applications where the soil quality criteria are reached within acceptable timeframes.

Dansk sammenfatning

I de seneste to til tre årtier er der publiceret adskillige studier, der viser, at planter effektivt kan rense forurenet jord og grundvand. Forventningerne til fytoremediering har således været store, men indtil nu er teknologien ikke blevet anerkendt eller taget i brug, kommercielt og regulatorisk, på linje med konventionelle jord og grundvands oprensningsteknologier.

Denne afhandling ser nærmere på fytoremediering og på hvorfor teknologien ikke har vundet anerkendelse og udbredelse. Formålet er at vurdere anvendeligheden af fytoremediering og at identificere mulige forhindringer for gennemførelsen. Herudover giver afhandlingen eksempler på og forslag til, hvordan disse forhindringer overvindes.

Første del af afhandlingen gransker litteraturen for data og erfaringer med anvendelse af fytoremediering. Den afdækker hvilke problemer, der kan opstå, samt hvor vi mangler erfaring og viden. Ydermere betragtes fytoremediering fra et mere fagteknisk perspektiv og opsætter en massebalance for et generelt plante-jord-system. På baggrund af litteraturgennemgangen og den opstillede massebalance vurderes anvendeligheden af fytoremediering for almene jord- og grundvandsforureninger.

Forskellige begrænsninger og videnshuller bliver udpeget. Fytoremediering er begrænset af jordens giftighed, planternes rodlængde samt lang oprensningstid. Herudover er der tydelig mangel på viden om forureningstypers skæbne i planter og planteprocesser, som påvirker optag, udskillelse og metabolisme. Denne type data er nødvendig for modellering af massebalancer.

Massebalancen afslører, at hvis forureningskomponenterne kan nedbrydes ($k_{nedbryning}$ ~ 0.01/dag eller højere), vil den primære fjernelse af forureningsmasse i jordmatricen ske ved aerob mikrobiel nedbrydning i rhizosfæren (rodzonen) og ikke ved optag i planter. Rhizonedbrydning af organiske stoffer anses derfor for at være anvendelig. Såfremt at den samlede fjernelse af forureningsmasse ved nedbrydning, fordampning og andre ukendte processer (planteoptag og udvaskning ikke taget i betragtning) kan negligeres, bliver den dominerende fjernelsesproces planteoptag. Sammenlignet med rhizonedbrydning er fjernelse ved planteoptag langsom og styres af volumen af jordmatricen samt jord til vand fordelingskoefficienten (K_d). For stoffer med høj K_d (f.eks. bly) kan fytoremediering tage overordentligt lang tid (>80.000 år).

Ekstraktionseffektiviteten af planter er proportional med jordvolumen, hvilket forklarer, hvorfor potteeksperimenter generelt undervurderer oprensningstiden. Anvendeligheden af fytoremediering for organiske stoffer med ringe aerob nedbrydning (f.eks. visse klorerede opløsningsmidler) men med relativt lav K_d (~10 L/kg) er sværere at vurdere på grund af manglende viden om deres skæbne.

Anden del af afhandlingen præsenterer to flowdiagrammer til vurdering af anvendeligheden af fytoremediering samt anvendeligheden af de enkelte fytoremedieringsteknikker. Disse skal ses som checklister af ting, som bør overvejes inden implementering af fytoremediering, og de er beregnet til at støtte og fremme beslutningsprocesser omhandlende fytoremediering. Yderligere fremhæver de behovet for giftighedsdata for terrestriske planter samt skæbne-, nedbrydnings- og massebalancestudier.

Det bliver vist, at de eksisterende toksicitetstests med terrestriske planter, som ikke involverer frøspiring, er svært gennemskuelige og er svære at sammenligne grundet manglende standardisering og test data afrapportering. Afhandlingen foreslår at den akutte piletræstest af Trapp et al. (2000) indstilles som kandidat til en standardiseret toksicitetstest med terrestriske planter, da den tillader kombinerede optag, metabolisme og giftighedsstudier. Et overblik over data genereret med den akutte piletræstest bliver præsenteret og sammenholdt med data genereret med standardiserede toksicitetstests på grønalger og andemad. Ingen af arterne kan klassificeres som følsommere end de andre. Det foreslås at der bliver etableret en database med toksicitetstestdata fra standardiserede tests på terrestriske planter, som ikke involverer frøspiring.

Skæbne og nedbrydning af trikloreten (TCE) i planter bliver vurderet med den akutte piletræstest, med klorid som indikator på dehalogenering. Piletræer og piletræer inokuleret med TCE co-metabolistiske mikroorganismer af bakterieslægten *Burkholderia cepacia* blev eksponeret for TCE i en tre-ugers periode. Ca. 96% af den tilførte TCE afdampede direkte fra testopløsningen og kun 4% blev taget op i planterne. Mindre end 3% af den tilførte TCE blev mineraliseret.

For at vurdere gennemførligheden af fytoremediering for et fluoridforurenet område, bliver optag af fluorid i planter studeret med den akutte piletræstest. Massebalancen viser, at piletræerne var i stand til enzymatisk at fjerne fluorid

fra rødderne ved lave jordkoncentrationer af fluorid. Ved høje jordkoncentrationer bliver enzymsystemet overbelastet og bryder sammen. Herved begyndte piletræerne at optage fluorid, og yderligere giftvirkninger blev observeret. Fytoremediering er vurderet ikke at være anvendeligt for grunden.

Arbejdet udført i denne Ph.D.-afhandling understreger at fytoremediering lejlighedsvist kan være anvendeligt. Dog er der flere begrænsninger, som besværligøre brugen. Fytoremediering er vurderet at være brugbart for næringsstoffer samt organiske forbindelser som kan nedbrydes aerobt. De fleste tungmetaller bindes stærkt til jordmatricen og er derfor ikke tilgængelige for planteoptag. Fytoremediering anses ikke som anvendeligt for pågældende forureningstyper. Førend fytoremediering kan blive anerkendt på linje med andre oprensningsteknologier, skal fytoremediering bevises effektivt ved afrapportering af succesfulde oprensninger i felten, hvor jordkvalitetskriterierne er nået indenfor en passende tidshorisont.

Table of contents

Abbreviations

BTEX	Benzene, toluene, ethylbenzene, xylene
CLP	Classification, labelling and packaging (within the framework of EU regulation 1272/2008)
DCAA	Dichloroacetic acid
DCE	Dichloroethylene
EDTA	Ethylenediaminetetraacetic acid
GMO	Genetically modified organisms
GW	Groundwater
ISO	International Organization for Standardization
K_d	Soil/water distribution coefficient
K_{ow}	Octanol-water partitioning coefficient
MTBE	Methyl tert-butyl ether
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyl
PCE	Tetrachloroethylene
PHC	Petroleum hydrocarbon
REACH	Registration, evaluation, authorisation and restriction of chemical substances (within the framework of EU regulation 1907/2006)
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TCAA	Trichloroacetic acid
TCE	Trichloroethylene
TCEtOH	Trichloroethanol
TeCP	Tetrachlorophenol
TNT	2,4,6-trinitrotoluene
TSCF	Transpiration stream concentration factor

1 Background and aim

Phytoremediation is a general term which covers a wide range of application of plants as a mean to treat contaminated air, soil, surface- or groundwater. Cunningham and Berti (1993) define phytoremediation as "*the use of green plants to remove, contain or render harmless environmental contaminants*". This PhD project, however, does not consider application of plants for treatment of surface water or airborne pollutants, dealing solely with remediation of soil- and groundwater. Trapp and Karlson (2001) define phytoremediation as the use of plants to clean contaminated soils. In this thesis the definition of phytoremediation by Trapp and Karlson (2001) is extended to denote the use of green plants and their associated microbes for *in-situ* treatment of contaminated soil- and groundwater.

The use of plants to deal with contamination in the environment is no new invention. For wastewater treatment it has been in use for at least 300 years (Cunningham and Berti, 1993; Cunningham et al., 1996). However, the application of phytoremediation for contaminated soil- and groundwater first emerged in the decade from the early 90'ties to early millennium (Anderson et al., 1993; Brown, 1995; Cunningham and Berti, 1993; Cunningham et al., 1995; Raskin et al., 1994; Salt et al., 1995; Trapp and Karlson, 2001). At that time the expectations and promises of phytoremediation were high and it was foreseen that the phytoremediation technology would get accepted quickly (Anderson et al., 1993; Gisbert et al., 2003; Moffat, 1995; Raskin et al., 1994; Salt et al., 1998). Today, two to three decades later, phytoremediation of polluted soil- and groundwater is still not widely applied or recognized commercially or in regulatory contexts (Gerhardt et al., 2017; Sharma and Pandey, 2014; Witters et al., 2012).

Before phytoremediation can become an established and accepted remediation technology there is a need to identify why the promises of phytoremediation was never fulfilled. The feasibility of phytoremediation for selected soil- and groundwater pollutants must be reassessed to re-evaluate the prevailing application and implementation methodologies. This thesis aims to elucidate some of these aspects and ultimately assess the feasibility of phytoremediation for common soil and groundwater pollutions. Special focus has been given to the following research questions:

- Identify knowledge gaps and obstacles, constituting a barrier for implementation of phytoremediation, by reviewing the existing literature and by observing the governing processes from a mass balance perspective. (**Paper I-IV**)

- Evaluate the uptake, metabolism and volatilization (fate) of selected soil- and groundwater pollutants in trees and their associated rhizosphere by use of experimental studies. (**Paper I and II**)

- Provide an overview of phytotoxicity data for higher terrestrial plants generated by the willow tree acute toxicity test to evaluate to which extent toxicity of pollutants hampers the use of phytoremediation. (**Paper I, III and IV**)

The research aims were reached by reviewing the existing literature and data reported for applied field and laboratory studies of phytoremediation. Also studies investigating processes and parameters describing plant-soil-substance interactions were carefully reviewed. The knowledge gaps identified made it possible to select some common soil- and groundwater pollutants for further investigations. These compounds were examined by the willow tree acute toxicity test by Trapp et al. (2000), modified for the purposes of the experimental designs. The dissertation puts together missing pieces within the three main applications of phytoremediation and determines the prerequisites, effectivity and obstacles of the technology. A flow chart presenting the topics of the thesis and the red line of this work is presented in Figure 1 and clarify how the scientific papers included fit and overlap the themes.

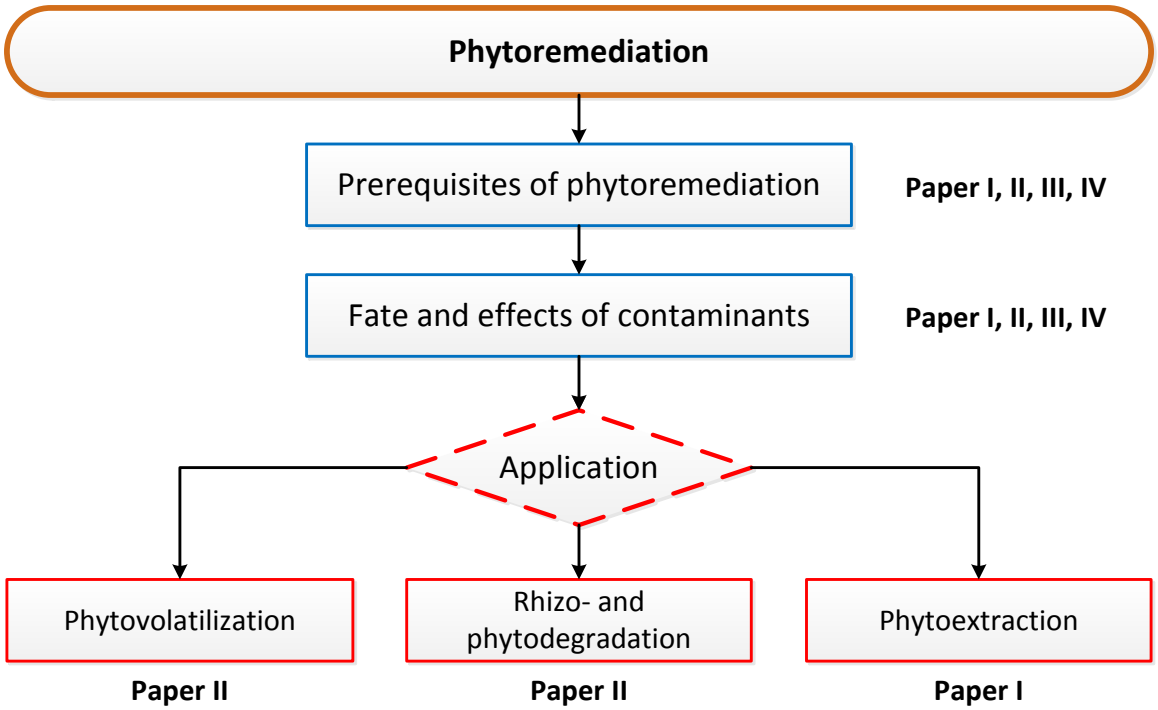

Figure 1: Flow chart presenting the topics of the thesis and how the scientific papers included fit and overlap the themes.

The papers I, II and IV present experimental work focusing on testing specific properties of selected compounds. These experimental studies are combined uptake/metabolism and phytotoxicity studies and provide pivotal insight in how to design phytoremediation experiments and applications. Paper III is a review of the phytotoxicity data generated with the willow tree acute toxicity test by Trapp et al. (2000).

2 Phytoremediation

Phytoremediation is a natural solar driven process (Flathman and Lanza, 1998; Susarla et al., 2002) which happens whenever plants get in contact with contaminated soil- or groundwater. In fact phytoremediation covers a wide range of processes, some well described in the literature, some not (McCutcheon and Schnoor, 2004). Important to note is that all of the processes may occur simultaneously and do not exclude each other. The governing processes are controlled by the physical-chemical properties of the contaminants taken up (Briggs et al., 1982; Schnoor, 1992). An overview of the many processes of phytoremediation is depicted in Figure 2.

The driver of all processes that relate to phytoremediation is the ability of plants to take up and transpire water, hereby creating a flux of water from the surrounding soil to the roots and taking up contaminants dissolved in the soil- and groundwater (Burken, 2004). The uptake of contaminants happens from the apoplast by diffusion, according to Fick's law, and passively with the transpiration stream or actively by use of energy (Ryan et al., 1988; Macnair et al., 1999). However, for most xenobiotics and non-essential elements the passive uptake is dominant (Shone et al., 1973; Trapp, 1995).

Contaminants in the xylem can be translocated upwards with the transpiration stream (Larcher, 1995a; Trapp and Matthies, 1998). During this process the contaminants may, depending on the physical-chemical properties, adsorb to the plant tissue and accumulate in roots, stem or leaves, or diffuse out of the system e.g. through the bark of the stem (Ma and Burken, 2004). Some contaminants might get transformed to other compounds or even get mineralized (Burken, 2004). The transpiration stream brings the water and contaminants from the roots via the stem to the leaves, from where the water is transpired through the stomata to the atmosphere (Larcher, 1995a). The evaporation of water from the leaves generate a negative pressure, driving the flow of water through the plant. Photosynthesis initiated in the chlorophyll of the leaves converts H_2O and atmospheric CO_2 to carbohydrates and O_2. The photosynthates are translocated from the leaves to consumer organelles (all living plant cells) or storage depots (roots, fruits, seeds) by means of the phloem, Figure 2 (Larcher, 1995b).

As noted in the grey box in Figure 2, there are many unknown processes which have not yet been described in the literature (Thakur et al., 2016). In the past decades an extensive effort has been made in order to describe and

understand many of these unknown plant processes and plant-environment interactions. However, the system is complex (Wenzel, 2009) and we are still far from understanding the full plant-environment aspects (Briggs et al., 1982; Gerhardt et al., 2009; Kleist and Luan, 2016; Kotak et al. 2007; Larcher, 1995c; Peñuelas and Llusià, 2001; Ryan et al., 1988; Vetterlein and Doussan, 2016).

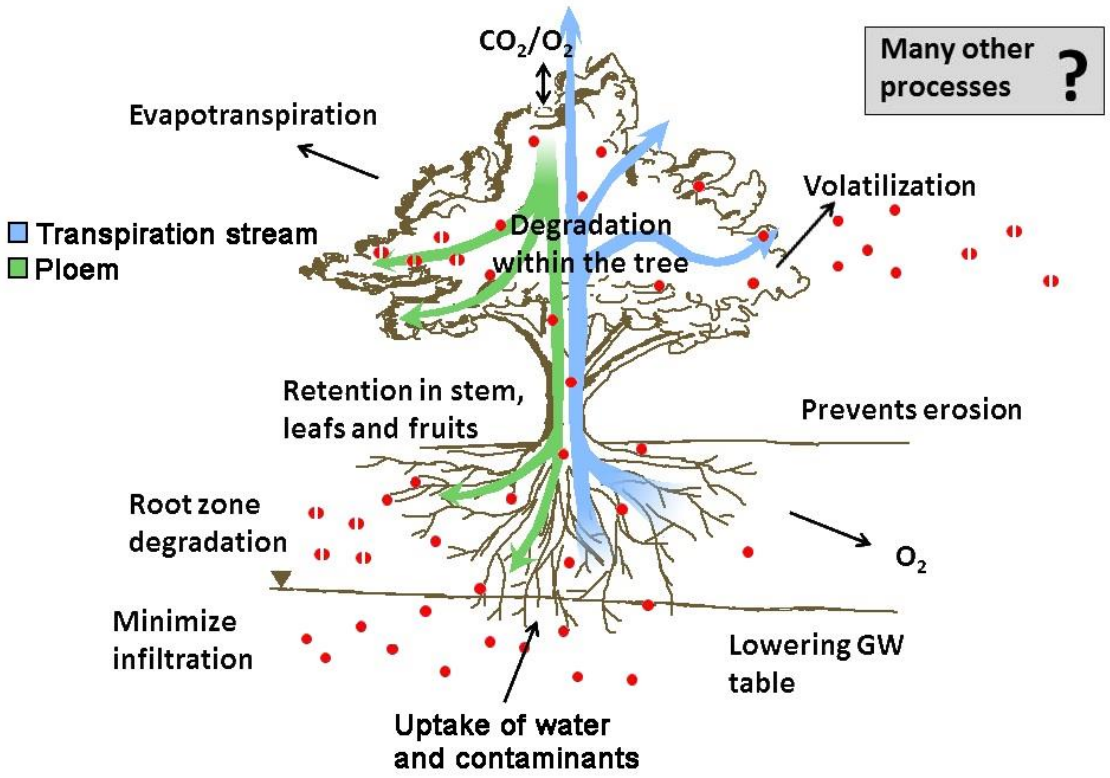

Figure 2: An overview of the main processes exploited in phytoremediation. All processes may occur simultaneously and do not exclude each other. Note that the real tree-environment-system is far more complex with many undescribed processes not presented here. Artwork by Sigurd Knarhøi Johannsen. Modified from Schnoor, 1997).

2.1 Applications of phytoremediation

The many processes taking place within and around the plant-environment-system, Figure 2, can be utilized to deal with various types of contaminated soil- and groundwater. Summarizing, the processes described in section 2 can be "boiled down" to three main applications of phytoremediation, namely: phytoextraction, phytovolatilization and rhizo- and phytodegradation. Which of the applications that is most suited or dominant for a particular site is highly dependent on the type of contamination present and site specific parameters. The three main applications are further described below together with

some additional benefits/uses of plants from a remediation perspective. Here possible applications of phytoremediation suggested in the literature are listed and described. Later, the effectiveness for various contaminants is quantitatively evaluated.

2.1.1 Phytoextraction

Phytoextraction is the use of plants to take up, translocate and store contaminants within the above ground tissue (Wensel, 2009). Phytoextraction is applicable for treatment of soils contaminated with non-volatile persistent pollutants such as heavy metals (Kumar et al., 1995), inorganic salts (Clausen et al., 2015 - Paper I) and radionuclides (Garbisu and Alkorta, 2001). As the plants are used to extract, up-concentrate and store pollution, this application requires continuous handling, harvesting and disposal or reuse, of the contaminated biomass produced. There are several suggestions in the literature for reuse or disposal of the contaminated plant tissue (Kovacs and Szemmelveisz, 2017).

2.1.2 Phytovolatilization

Direct or just phytovolatilization is the evaporation of contaminants with the transpiration stream (Limmer and Burken, 2016; Trapp and Karlson, 2001). Indirect phytovolatilization is the increased volatilization flux of contaminants from the subsurface caused by transpiration of soil pore water and subsequent enhanced diffusion in gas pores (Limmer and Burken, 2016). Phytovolatilization is applicable for contaminants with a log K_{OW} < 3.5-5 (Limmer and Burken, 2016; Trapp and Karlson, 2001) and with a Henry's law constant >> 10^{-5} (dimensionless) (Trapp and Karlson, 2001).

2.1.3 Rhizo- and phytodegradation

In rhizo- and phytodegradation plants and their associated rhizospheric and endophytic microbes are utilized to capture and metabolize organic pollutants (Wensel, 2009). Rhizo- and phytodegradation is applicable for all available organic contaminants which are not persistent (McCutcheon and Schnoor, 2004), e.g. petroleum hydrocarbons (PHC), chlorinated solvents, some pesticides (Newman and Reynolds, 2004) and excess nutrients (Cunningham et al., 1995).

2.1.4 Other applications

Besides the main applications of phytoremediation, described above, plants and their associated microbes have a long list of beneficial properties which can be utilized for environmental purposes. These applications will not be

further discussed in this thesis but the most important are still worth mentioning in short terms.

Hydraulic control and vegetation cover: Vegetation transpires water thus minimizing infiltration of rainwater and thereby leachate from underlying subsurface pollution. Plants can be used to reduce the cost of, or in some cases avoid the need of, pumping (Trapp and Karlson, 2001). Some areas may also be reclaimed for various purposes, e.g. grazing, by desiccation of plants. Vegetative cover is used to stabilize the soil and contaminants in affected areas (Gyssels et al., 2005; Trapp and Karlson, 2001). The plant roots stop soil erosion minimizing distribution of contaminated dust. As a mean of water quality protection of streams, vegetation buffer zones have been utilized to limit the washout of nutrients, particulate matter, pesticides and more from agricultural land to surface waters e.g. streams draining the farmland (Correll, 1996). Further, vegetative cover improves the aesthetic beauty of the otherwise futile site (Trapp and Karlson, 2001). Lastly, the bacterial activity in the plant root zone is promoted (Cunningham and Berti, 1993). The rhizospheric bacteria benefit from the sugar rich plant exudates released from the roots and the nutritious stream of water passing by the roots due to the transpiration or "pumping" of the plants (Glick, 1995; Whipps, 2001). Rhizospheric soil contain one to three orders of magnitude more bacteria than unvegetated soils (Anderson et al., 1994; Barbyshire and Greaves, 1967).

Rhizofiltration: Is sorption of chemicals or precipitation of the contaminants in the rhizosphere. The method is mainly applicable for heavy metals and lipophilic organic compounds (Trapp and Karlson, 2001; McCutcheon and Schnoor, 2004).

Land farming: In land farming contaminated soil or sludge is mixed into the topsoil of a fertilized field with fast growing crops like alfalfa or grass (Trapp and Karlson, 2001). The aerated and fertilized conditions increases the degradation of most organic pollutants e.g. PHCs.

Bringing oxygen to the subsurface: By taking up and transpiring water, plants draw down the groundwater table thus simultaneously drawing down atmospheric air containing oxygen, which is essential for the mineralization of most organic pollutants (Chiang et al., 1989; Kristensen et al., 1995). An additional effect of water uptake on oxygen transport and delivery to the subsurface is enhanced diffusion. Diffusion of oxygen in air is more than one thousand times faster than diffusion in water (Nazaroff and Alvarez-Cohen, 2001) so by removing water from the soil pores oxygen is allowed to diffuse

more easily into the rhizosphere. Lastly, many swamp species are capable of pressurized gas transport between the atmosphere and the subsurface through root and stem aerenchyma (Grosse et al., 1992). This allows enhanced oxygen transport to the rhizosphere and subsequently enhanced aerobic degradation.

Air filtration: Most plant leaves, except species from arid regions, are designed with a high surface to volume ration, allowing high rates of gas exchange and transpiration (Larcher, 1995c). The high gas exchange rates enable the plants to efficiently remove many environmentally problematic air pollutants (Hill, 1971) and the large area promotes particle decomposition at the leaf surface's (Smith and Jones, 2000). Further, the leaf surface of many plant species is covered by a waxy layer which can act as a refuge for lipophilic compounds settling there (Eglinton and Hamilton, 1967).

2.2 Prerequisites of phytoremediation

Despite the many possible uses of phytoremediation, plants and their application in remediation of contaminated soil and groundwater have several prerequisites or limitations which have to be considered before implementation. The maximum rooting depth of plants greatly varies from species to species and region to region (Canadell et al., 1996). For effective application of phytoremediation, except hydraulic control, the pollution must be located well within the maximum rooting depth of the chosen plant species. For most types of vegetation this limits phytoremediation to sites with shallow contamination, no deeper than three meters below surface (mbs) or in some cases within one mbs (Canadell et al., 1996; Ghosh and Singh 2005). Candell et al. (1996) report an average maximum root depth of 3 mbs for temperate deciduous trees, 3.2 mbs for temperate coniferous trees (temperate coniferous trees from arid areas excluded) and 2.5 mbs for temperate grassland species. If the contamination is within reach of the plants several issues have to be addressed. Firstly, phytoremediation is limited by toxicity of the contaminants to the plants applied (Clausen and Trapp, 2017 - Paper III; Trapp and Karlson, 2001). Phytotoxicity and the testing of phytotoxicity of chemicals is further discussed in chapter 5, but it depends on the physical-chemical properties of the chemicals present, site specific properties (temperature, soil organic carbon, and more), pH and plant species (Clausen and Trapp, 2017 - Paper III). Besides the chemical toxicity to plants, climate impacts on the plant species applied are crucial to consider. It may seem obvious that introducing exotic plant species to temperate climate zones and vice versa often

result in failure and plant death. This fact is however often not considered when looking for efficient phytoremediation species (Fischerová et al., 2006; Lombi et al., 2001; Salido et al., 2003; Tu et al., 2002). Secondly, phytoremediation may be limited by transpiration. Plants mainly extract chemical components dissolved in the water transpired, meaning that chemicals not present in the water phase (insoluble components or compounds sorbing too strongly to the soil matrix) will not be taken up. Uptake of e.g. non-aqueous phase liquids may still be possible or by diffusion. The rate at which a dissolved substance enters a plant is, if no active exclusion exists, directly proportional to the amount of water transpired and the soil water concentration (Clausen et al., 2015 - Paper I). Plants with high transpiration rates (high growth) are thus preferred for phytoremediation purposes, but extraction rates still tend to limit the remediation efficiency (Trapp et al., 2014; Clausen et al., 2015 - Paper I). The often long remediation time may be a limitation in itself (Trapp et al., 2014).

3 Experiences of phytoremediation

Many studies have reported results and experiences from laboratory and field experiments as well as for some full scale applications of phytoremediation (Gerhardt et al., 2017; Vangronsveld et al., 2009). The following scrutinizes the existing knowledge within applicability of phytoremediation for various environmental pollutants which is later utilized in the assessment of the feasibility of the technology.

3.1 Trace elements and radionuclides

Most heavy metals, or trace elements and radionuclides found in soil and groundwater are non-volatile. Trace elements and radionuclides cannot be degraded except for radioactive decay. Phytovolatilization and rhizo- and phytodegradation are thus not suited for the treatment of these compounds, leaving only phytoextraction as a suitable phytoremediation approach.

Phytoextraction of trace elements have had increasing focus over the years (Ghosh and Singh, 2005; Huang et al., 1997; Lombi et al., 2001; Mahar et al., 2016; Midhat et al., 2017). Much of the research described covers laboratory pot experiments of extraction efficiency of hyper accumulating plant species and methods for increasing the plant available fraction of metals. This, at the same time highlights where to find the limitations of phytoextraction. Trace elements and radionuclides bind strongly to soil particles and organic matter in the soil matrix. Typical values of the distribution coefficient between solid matter and water, K_d, are in the order of 4400-23,300 (L/kg) for zinc, 50-1,000 (L/kg) for nickel, 500-1000 (L/kg) for copper and 8-4000 (L/kg) for cadmium (cationic metal species) (Jensen, 2000; USEPA, 1999). The anionic trace elements (e.g. arsenic and chromium) are sorbed to a less degree due to the negative charge of the soil particles (Jensen, 2000). The high K_d-values result in high sorption rates making the trace elements poorly available to the plants. This greatly minimizes the extraction efficiency. Estimated remediation timeframes have been reported in the order from a couple of growth seasons (Tangahu et al., 2011), two to three decades (Brown et al., 1995; Ostman, 1994) to more than 80,000 years Trapp et al. (2014). Algreen et al. (2014) report extraction rates of cadmium, copper, nickel and zinc by willows at a former sewage sludge disposal in Valby, Denmark, in the order of 0.1-0.5% over 10 years, indicating a long remediation timeframe. The major bottleneck within phytoextraction is to improve the availability of the trace elements and radionuclides thereby enhancing the extraction efficiency.

The most common strategy utilized to enhance the mobility of the metals is addition of chelating agents, e.g. synthetic aminopolycarboxylic acids such as ethylene diamine tetraacetic acid (EDTA), to the soil matrix (Evangelou et al., 2007; Lombi et al., 2001) - A concept referred to as "*chemically assisted trace element phytoextraction*" (Evangelou et al., 2007; Vangronsveld et al., 2009). The addition of chelates can increase the water soluble trace element fraction, for some metals more than 5000 fold, at the same time increasing the plant uptake. The uptake of trace elements to the plants is however not linearly correlated with the plant available trace element concentration in the soil matrix (Evangelou et al., 2007). Despite these optimistic findings, no successful full scale field remediation has been reported yet. One of the earliest field scale applications of phytoextraction reported was by McGrath et al. (1993). They reported zinc uptake in *Thlaspi caerulescens* of 2000-8000 mg Zn/kg dry weight, corresponding to 40 kg Zn/ha in one growing season, concluding a sufficient remediation time (time to reduce zinc concentrations below the soil quality criteria of 300 mg/kg) of approximately 9 growing seasons. Remediation of the site should thus have been completed more than a decade ago. Still no successful phytoextraction remediation studies are reported. As, mentioned, Trapp et al. (2014) used a simple mathematical model by Algreen et al. (2014) for prediction of the treatment time of phytoremediation at a trace element contaminated site in Hungary. The model used measured bioconcentration factors in plants at the site and estimated a remediation time of more than 80,000 years. This value is strikingly high but testifies that phytoremediation of trace elements with non-hyperaccumulating species will be a long-term operation.

Hyperaccumulating plants are defined as species that accumulate, as dry weight, more than 100 mg/kg cadmium, 1000 mg/kg nickel, cobalt, copper, lead or selenium or 10,000 mg/kg zinc or manganese (Baker et al., 2000; Brooks, 1998). A great effort has been made to map and identify potential hyperaccumulating plant species and other plant types (e.g. fast growing high transpiring plants) with potential for phytoextraction (Chehregani et al., 2009; Lange et al., 2017; Saladin, 2015). An example is the Chinese furn *Pteris vittata* which accumulated up to 4 g/kg (dry weight) at a former wood preservation site in Hillerød, Denmark, in one growth season.

The major issue of the hyperaccumulating plants is that they typically are slow growing species with extraction rates limited by low transpiration (Cunningham and Ow, 1996). A major effort has been to create transgenic plants with specific properties, allowing higher extraction rates or increased survival

rate (Eapen and D'souza, 2005; Ibañez et al., 2016). Examples are Song et al. (2003) who demonstrated increased uptake and resistance in a genetically modified *Arabidopsis thaliana* to lead and cadmium, Verma et al. (2016) showed high resistance of a transgenic *A. thaliana* to arsenic as a result of enhanced arsenic volatilization and Gisbert et al. (2003) observed enhanced tolerance and accumulation of lead in a genetically modified *Nicotiana glauca*.

Summarizing, the focus within the field of phytoextraction of trace elements is to find naturally and genetically modified plant species capable of hyperaccumulating pollutants (Chandra et al., 2017; Pollard et al., 2014; Viktorova et al., 2016) as well as finding measures to improve the fraction of contaminants available to plants (Sheoran, 2016; Wang et al., 2017).

3.2 Organics and nutrients

Organic pollutants cover a wide range of contaminants with very different environmental behaviour (Burken, 2004). Some compounds like the chlorinated solvents, e.g. TCE, are hydrophobic and volatile (Jackson and Dwarakanath, 1999), some compounds like gasoline components such as alkanes and aromates are easily degraded under aerobic microbial activity while others are persistent organic pollutants (POPs) potentially staying in the environment for decades (Trapp and Legind, 2011). Best known examples are dichlorodiphenyltrichloroethane (DDT), the poly chlorinated biphenyls (PCB) and many of the polycyclic aromatic hydrocarbons (PAH). The diverse environmental behaviour of the organic compounds makes it pivotal to assess which of the phytoprocesses that govern the fate of the contaminants present. Concerning volatile or semi-volatile substances extraction and subsequent storing of the pollutants in the biomass is of minor interest. Only for some of the persistent non-volatile compounds like the long chained PAHs (Singer and Finnerty, 1984) this may be of interest. From a phytoremediation perspective, the more interesting processes are rhizo- and phytodegradation and phytovolatilization.

Rhizo- and phytodegradation of organic pollutants have received extensive attention (Burken, 2004; Feng et al., 2017; Schnoor et al., 1995). To find the best suited plants for phytoremediation of oil products, Ikeura et al. (2016) screened 33 plant species for their ability to clean hydrocarbon contaminated soils. They found that many species were limited by soil toxicity. Also, numerous pot experiments have been conducted with various organic pollutants

(Basu et al., 2015; Kaimi et al., 2007; Newman et al., 1997; Smith et al., 2007 and more) many of these with promising outcomes.

Phytoremediation of organics has also been conducted at field scale. Newman et al. (1999) studied uptake and mineralization of TCE in hybrid poplars. They observed remarkable efficient removal in a constructed artificial aquifer. More than 99% of the added TCE was removed at the planted plots compared to 33% for unplanted controls during a three year period. Less than 9% was volatilized to the atmosphere. These finding are in contradiction with recent studies by Clausen et al. (2017a) (Paper II) and Schöftner et al. (2016), which found that willows (*S. viminalis*) were not capable of significant TCE degradation under controlled laboratory conditions. Other studies report microbially enhanced phytoremediation of TCE. Weyens et al. (2009) did some of the first field scale inoculations of hybrid poplars (*Populus trichocarpa*×*Populus deltoids*) with a TCE degrading strain of *Pseudomonas putida* W619-TCE and observed a 90% reduction of phytovolatilization to the atmosphere. Recently and remarkably, Doty et al. (2017) observed complete mineralization of TCE by poplars inoculated with the natural endophyte *Enterobacter* sp. strain PDN3 within 3 years.

TCE is one of the best examined compounds with respect to phytovolatilization rates. Reported values for TCE in poplar trees ranges from 0.04-2.88 μmol m^{-2} d^{-1} (stems) and between 0.09-0.13 μmol m^{-2} d^{-1} (leaves) (Doucette et al., 2013; James et al., 2009; Newman et al., 1999). The volatilization rates through tree trunks greatly depends on plant age/size as young/small trees have a much higher surface to volume ratio and thus a higher potential for diffusive volatilization.

Sun et al. (2011) studied the PAH degradation potential of alfalfa (*Medicago sativa L.*) and tall fescue (*Festuca arundinacea Schreb.*) under field conditions. They observed a 19.9% reduction of total soil PAH for monoculture applications and 30.5% reduction when mixing the plants. The control plot (unplanted plot) did not show any reduction in the total soil PAH concentration during the 7 months treatment.

For benzene, toluene, ethylbenzene and xylene (BTEX) and other petroleum hydrocarbons (PHC) rhizo-and phytoremediation have been applied several times at field scale. Siciliano et al. (2003) reported a 30% reduction of the soil concentrations during a field scale study running from July 1998 to February 2000. Simultaneously, the unplanted plot showed a 15% reduction of the total PHC concentration. Similarly, Nedunuri et al. (2000) observed at a

field site a soil concentration reduction of PAH of approximately 69% for rye grass (*Lunaria annua*), 61% reduction for St. Augustine grass (*Stenotaphrum secundatum.*) and 63% reduction of Sorghum (*Sorghum biocolor*) over a two year duration (Unplanted control had 57% reduction). Likewise, Gurska et al. (2009) observed more than 60% reduction in the, high molecular weight fraction, soil concentration of a refinery hydrocarbon waste at a site in southern Ontario, Canada (30% for unplanted controls). The study was done over a 3-year period with a variety of plant species (*L. perenne, Festuca arundinacea, Secale cereale* and *H. vulgare*) inoculated with two plant growth promoting rhizobacteria (*Pseudomonas sp*. strain UW3 and UW4). Similarly but slightly faster, Ji et al. (2004) reported 96% removal of heavy hydrocarbons within two years of treatment by mature reeds (*Pheagmites sp.*) (no unplanted plot included in the experiment).

Hong et al. (2001) studied uptake and fate of MTBE in hybrid poplars (*Populus deltoides x nigra DN-34*, Imperial Carolina) in a laboratory experiment and in the field. They concluded that MTBE is readily taken up and volatilized through leaves and stems and that the hybrid poplars were capable of hydraulic containment and remediation of the MTBE plume. These findings were confirmed by Ma et al. (2004) which conducted a laboratory study of MTBE phytovolatilization by the same species. Trapp et al. (2003) and Ramaswami et al. (2003) showed that MTBE degradation in plants and their rhizospheres is insignificant and that the main loss is phytovolatilization.

Phytoremediation of explosives have also received quite some attention (Via and Zinnert, 2016) and the most commonly encountered of these in the environment are 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). van Dillewijm et al. (2007) studied TNT degradation by hydroponic grown maize (*Zea mays*) and broad beans (*Vicia faba*). In the laboratory tests they observed 32% and 38% degradation of TNT after one week, respectively and 3% degradation in the control experiment. They also conducted a field scale experiment with corn (Z. mays) where 96% of the TNT was degraded in 60 days compared to 0% degradation at the unplanted lots.

Phytoextraction of the very lipophilic (log $K_{OW} > 4$) compounds, e.g. some PAHs and the PCBs, have previously been considered of little importance. Transgenic plants may provide a solution for these compounds (Hernández-Vega et al. 2017) but also a few studies have demonstrated significant uptake by specific plants species such as zucchini and pumpkin (Schwitzguebel, 2015) and microbially enhanced phytoremediation has also been reported.

Teng et al. (2010) inoculated alfalfa (*Medicago sativa L.*) with fungus (*Glomus caledonium* 90036) and bacterium (*Rhizobium meliloti*) and observed 43.5% reduction of PCB's at field scale. No degradation was observed for the unplanted control plot. Lin et al. (2016) discovered that the uptake of at least some (di-n-butyl phthalate) lipophilic organic compounds to pumpkins (*Cucurbita moschata*) can be explained by enzymatic processes described by the Michaelis-Menten equation. In general, the basic processes involved in detoxification, transformation, and translocation and ultimately mineralization of organic contaminants in plants and their rhizospheres are not well understood (Hurtado et al., 2016; Sulpice and McKeown, 2015).

Phytoremediation of excess nutrients has proved to be highly applicable (Pilon-Smits, 2005; Schnoor et al., 1995). Plants are widely used as buffer zones along rivers and streams, reducing washout of nutrients from e.g. agriculture fields. Plants like willows are also used for cleaning of urban wastewater (Shi et al. 2016).

3.3 Inorganic salts

Phytoremediation of inorganic salts is not as well investigated and developed as heavy metals and organic contaminants. Potassium cyanide (KCN) may be the best studied salt to date. Larsen et al. (2014 and 2015) studied toxicity, uptake and metabolism of KCN in hybrid willows (*Salix viminalis x schwerinii*). They observed a strong correlation between uptake and toxicity and found that plant roots were able to metabolize KCN at a rate of 10 mg CN/kg fresh weight/h. Besides KCN the focus has mainly been on recovery of agricultural soils and on how to protect crops at high salinity soils (Ashraf et al., 2010). Qadir et al. (2005) did a review of sodium extraction by non-hyper accumulating plants in pot experiments. Kallar grass (*Leptochloa fusca Kunth*) had the highest extraction efficiency ranging from 37-76% within 12-15 month. Ravindran et al. (2007) studied phytoextraction of sodium chloride by halophytes in a 120 days field experiment. The two salt accumulators *Spartina maritima* and *Sesuvium portulacastrum* showed the highest extraction rates of 504 and 474 kg/ha, respectively. The uptake of chloride in willows has been studied by Trapp et al. (2008). They found that the uptake of chloride could be described by the Michaelis-Menten equation for enzymatic removal. At low salt concentrations (<0.4 g/L) the willows were capable of pumping out most chloride and avoid toxic effects but at high concentrations (>0.4 g/L) their enzymatic system collapsed, taking up chloride passively with the transpiration stream. Clausen et al. (2015) (Paper I) conducted a sim-

ilar experiment with sodium fluoride. They observed similar results on a molar basis, which confirms the existence of an outwards directed pump for removal of these ions from plant roots. This most likely hampers or prolongs phytoremediation of many inorganic salts. The consequences of this enzymatic mechanism were studied by Bauer-Gottwein et al. (2008) for the Okavango island system, Botswana, where salt concentrations have increased to phytotoxic levels.

4 Mass balance and potential feasibility of phytoremediation

To assess the feasibility of phytoremediation from a more technical perspective, a generic mass balance can be established. Equation (*1*) describes the total contaminant mass removed from the system, accounting for the mass extracted by the plants, mass leached to other soil compartments and contaminant mass degraded or removed by first-order processes.

$$\frac{dm}{dt} = -Q * C_W - L * C_W - k_L * m \qquad (1)$$

where m/dt is the change of contaminant mass in the soil (mg), m is the contaminant mass in soil (mg), t is the time (day), Q is the transpiration of the plants (L/day), C_w is the contaminant concentration in soil pore water (mg/L), L is the flow of water leaching (L/day) and k_L is the first-order lumped loss rate constant (1/d).

C_w can be expressed by the soil concentration (C_s) and the soil/water-distribution coefficient ($C_w = C_s/K_d$) (Trapp and Matthies, 1998). Re-expressing equation (*1*) in terms of changes in contaminant concentration in the soil, the following expression is obtained.

$$\frac{dC_S}{dt} = \overbrace{-\frac{QC_S}{M_S K_d}}^{\text{Plant uptake}} \underbrace{-\frac{LC_S}{M_S K_d}}_{\text{Leaching}} \overbrace{-k_L C_S}^{\text{Other losses}} \qquad (2)$$

where C_s/dt is the change of contaminant concentration in soil (mg/kg wet weight (w.w.)), C_s is the contaminant concentration in soil (mg/kg w.w.), M_s is the soil mass (kg w.w.) and K_d is the soil/water distribution coefficient (L/kg).

Equation (*2*) highlights that five variables are controlling the removal of contaminants, besides the soil concentration, namely the transpiration rate of the plants (Q), the site specific leaching flow (L), the mass of contaminated soil (M_s), the soil/water distribution coefficient (K_d) and the lumped first-order removal rate (k_L). It is important to note that the "other losses" term is by far the largest of the three terms for degradable compounds (approximately three orders of magnitude with a $k_L \sim k_{deg}$ of 0.01 day^{-1}) when using standard default

input-values, Table 1. This indicates that phytoremediation is far more efficient for compounds which can be degraded in the rhizosphere (not limited by plant uptake rates). When observing the other terms removal of contaminants is slow for high soil matrix volumes and for low transpiring plants. K_d has to be small and k_L high for efficient removal.

Table 1: Input parameters for mass balance calculations for various scenarios.

Scenario	**C_0 [a, *] (mg/kg)**	**Q [b] (L/m²/day)**	**L [a] (L/m²/day)**	**M_s [a, *] (kg)**	**K_d [c,d] (L/kg)**	**k_L [a] (day^{-1})**
Default	100	0.8	0.1	5000	10	0.01
Simulations with k_L~k_{deg} = 0						
No deg.	100	0.8	0.1	5000	10	0
High Q	100	8	0.1	5000	10	0
High L	100	0.8	1	5000	10	0
Low M_s	100	0.8	0.1	500	10	0
High K_d	100	0.8	0.1	5000	20000	0

a) Estimated realistic values; b) Larcher (1995c); c) ACD/I-lab prediction; d) USEPA (1999); *) wet weight.

By assuming that both Q and L are constant in time the analytical solution to equation (*2*) is given by:

$$C_s(t) = C_0 e^{\left[\left(\frac{-Q-L}{M_s K_d} - k_L\right)t\right]} \qquad (3)$$

where $C_s(t)$ is the contaminant concentration in the soil (mg/kg w.w.) at time t (day) and C_0 is the initial contaminant concentration in the soil (w.w.).

Table 2: Contaminant concentrations in soil (mg/kg w.w.) after one year of phytoremediation. Input values for the various scenarios are outlined in **Table 1**.

t =1 year	**Default**	**No deg.**	**High Q**	**High L**	**Low M_s**	**High K_d**
$C_s(t)$ (mg/kg)	0.0	59.0	0.8	35.0	0.5	99.9

Equation (*3*) allows prediction of the contaminant concentration in the soil at any time. Performing a simple sensitivity analysis for a one-year simulation changing the parameters one by one as outlined in Table 1, it is observed that the most important parameter is the lumped first order removal rate. k_L thus introduces a major source of uncertainty to the calculations. For most trace elements and radionuclides k_L may be set to zero and for many organic compounds k_L can be approximated by the microbial aerobic degradation rate

(k_{deg}). Degradation in the rhizosphere dominates the removal at k_L~k_{deg} = 0.01 (day^{-1}) but assuming no degradation (k_L~k_{deg} = 0), e.g. for trace elements, the loss of contaminants from the soil is controlled by the transpiration rate, the soil mass (w.w.) and K_d. Table 2 presents the contaminant concentrations in soil (mg/kg w.w.) after one year of phytoremediation for the scenarios outlined in Table 1.

The ultimate goal of the mass balance modelling is to estimate the overall removal rate of contaminants from the soil matrix and thus predict an approximate remediation time. Rearranging equation (*3*), the approximate removal time (t) is expressed:

$$t = \frac{\ln\big(C(t)\big) - \ln(C_0)}{\frac{-Q-L}{M_S K_d} - k_L} \qquad (4)$$

Equation (*4*) provides the approximate remediation time for the contaminant concentration in the soil (mg/kg w.w.) to reach a certain threshold level.

As an example, a site in northern Sealand, Hillerød, Denmark, is contaminated with heavy metals after decades of wood impregnation activities. The site holds concentrations of arsenic of at least 1,600 mg/kg (w.w.) (Thomas, 2015). The Danish soil quality criterion for arsenic is 20 mg/kg (w.w.) (DKEPA, 2014). With default input values of Q, L and M_s, Table 1, K_d for arsenic of 13,100 (Sauvé et al., 2000) and no degradation k_L~k_{deg} of 0 day^{-1}, the remediation time to reach the Danish soil quality criterion is little less than 900,000 years.

To assess the general remediation horizons for some common soil and groundwater pollutants Table 4 presents estimated typical values of K_d, k_{deg} and their resulting estimated remediation times for various compounds. It is important to note that the remediation times (t) obtained by equation (*4*) are not accurate but rough estimations solely fit for initial assessment of the feasibility. More complex models are needed for proper estimations of the remediation time. Table 4 also summarizes the potential feasibility of phytoremediation for the substances presented. Low K_d values are observed for salts, explosives and nutrients and high K_d values for trace elements, PAH, radionuclides and some organic compounds. The approximated removal times are expected times required to reach contaminant concentrations in the soil of 0.1 mg/kg w.w. and are based on the default input parameters provided in Table 1 (except for K_d and k_L). It is observed that removal times for highly degradable compound groups (k_{deg} > 0.1) are fast (<1 year). However, these values are

not realistic as they assume aerobic degradation. In reality, fast degrading compounds will be limited by oxygen deficiency, why the short removal times should be seen as a potential best case scenario. Inserting the microbial anaerobic degradation constant instead of the aerobic rate constants may give a more realistic (worst case scenario) assessment for these compounds. For the calculation for chlorinated solvents of which the aerobic degradation rates are controversial k_L of 0 was applied. This might be too conservative and the remediation time can thus be regarded as a worst case scenario. The assessments of Table 4 are conducted on the basis of the knowledge presented in chapter 3 and 4 and solely reflect the author's point of view. Phytoremediation of trace elements is not feasible. They are too strongly sorped to the soil matrix and cannot be degraded. In very few cases, e.g. for Cd, there might be hyper accumulating plant species available but the time horizon is long. For the >three-ring PAHs the story is the same but for the lower-ring PAH's phytoremediation may be applicable. The chlorinated solvents are controversial but data presented in this thesis (Clausen et al., 2017a – Paper II) suggests that rhizo- and phytodegradation is not feasible. Phytovolatilization is applicable but will be time demanding. For readily degradable organic substances (PHC, BTEX, explosives) and nutrients phytoremediation may be feasible. Inorganic salts are not sorped to the soil matrix but are not considered feasible for phytoremediation as data suggests limited plant uptake (Clausen et al., 2015 – Paper I).

Table 3: Estimated typical values of K_d, k_{deg} and the approximate remediation time together with the assessment of potential feasibility of phytoremediation for common soil and groundwater pollutants. The approximate time is estimated by equation (*4*) with the default values of Table 1 (K_d and k_{deg} not included) with target concentration of 0.1 mg/kg (w.w.). The evaluation is based on the state of the art knowledge presented in chapter 3 and 4.

Group	Compound	Typical K_d (L/kg)	Typical k_{deg} (d^{-1})	Approx. removal time (year)	Phyto-extraction	Phyto-volatilisation	Rhizo- and phyto-degradation	Comments
Trace elements /radionuclides	As	13,100[a]	-	>1.8 million	(X)	(X)	-	
	Se	44,000[a]	-	>6 million	(X)	(X)	-	
	Cd	2,800[a]	-	390,000	(X)	(X)	-	
	Pb	170,000[a]	-	24 million	(X)	(X)	-	
	Sr	130[a]	-	18,000	(X)	(X)	-	
PAH	Naphthalene	100[b]	0.31[c]	(<1)	(X)	-	X	Deg. limited by O_2
	Benzo(a)pyrene	3600[b]	10^{-3} [c]	25	(X)	-	(X)	Deg. limited by O_2
Cl-solvents	Trichloroethylene	10[b]	ND	1000	-	X	(X)	Worst case (No deg. assumed)
PHC	Pentane	86[b]	0.42[d]	(<1)	-	(X)	**X**	Deg. limited by O_2
	Hexadecane	129,000[b]	0.03[e]	(<1)	-	-	(X)	Deg. limited by O_2
BTEX	Benzene	19[b]	0.1[f]	(<1)	-	X	**X**	Deg. limited by O_2
	Toluene	34[b]	0.23[g]	(<1)	-	X	X	Deg. limited by O_2
Explosives	TNT	2[b]	0.17[h]	(<1)	(X)	-	**X**	Deg. limited by O_2
Other organics	HCDD	18,600[b]	$6.9 \cdot 10^{-6}$ [i]	3600	-	-	(X)	
	MTBE	5[b]	$3.1 \cdot 10^{-3}$ [j]	8	-	X	(X)	Deg. limited by O_2
	DDT	2000[b]	$4.3 \cdot 10^{-3}$ [k]	6	(X)	-	(X)	Deg. limited by O_2
Salts	NaF	<1[l]	-	140	(X)	-	-	
	KCN	0.9[b]	0.5[m]	(<1)	(X)	-	X	Deg. limited by O_2
Nutrients	Ammonia	<1[l]	-	140	**X**	-	**X**	

X indicates highly applicable, X applicable, (X) may be applicable, – not applicable and ND no data; a) Sauvé et al. (2000); b) ACD/I-lab prediction. K_d-values are estimates from octanol-water partitioning coefficients (K_{OW}) according to Karickhoff (1981) with a reference soil containing 5% organic carbon; c) Park et al. (1990); d) Garnier et al. (1999); e) Noordman et al. (2002); f) Chiang et al. (1989); g) Liste et al. (2002); h) French et al. (1998); i) Sinkkonen and Paasivirta (2000); j) Metcalf et al. (2016); k) Fang et al (2012); l) K_d is small. For $K_d \rightarrow 0$ $t \rightarrow 0$. For calculations K_d was set to 1; m) Dumestre et al. (1997). Brackets for removal estimations indicate overestimation of the true degradation. TNT is 2,4,6-trinitrololuene, HCDD is 1,2,3,4,7,8-Hexachlorodibenzodioxin and, MTBE is methyl *tert*-butyl ether, DDT is 1,1'-(2,2,2-Trichloroethane-1,1-diyl)bis(4-chlorobenzene), NaF is sodium fluoride and KCN is potassium cyanide.

5 Testing strategy for applicability of phytoremediation

When scrutinizing the work done within the field of phytoremediation, it is evident that common guidelines in phytoremediation are not applied, chapter 3. Harmonizing procedures before and during the application of phytoremediation may help to overcome fallible implementation. The processes involved in phytoremediation are complex (Wenzel, 2009) and from a decision makers perspective it may seem too complicated to assess and secure a successful outcome within an acceptable timeframe. The knowledge gained in the first part of this thesis indicates for which substances phytoremediation might be feasible and what to consider before implementation. Summarizing, three of the key findings obtained in chapter 3 and 4 are:

- Remediation time of phytoremediation is long. Often longer than one or two decades.
- Phytoremediation is controlled by k_{deg} and/or K_d and knowledge about phytotoxicity to terrestrial plants is a prerequisite.
- The experiences with phytoremediation, from field and laboratory, build upon a variety of unique test designs.

Before implementing phytoremediation at field scale, these three issues have to be considered in order to successfully assess the feasibility. In the following the knowledge learned in chapter 3 and 4 is utilized to propose a simple guideline for the assessment of the applicability of phytoremediation.

5.1 Applicability decision-support flow chart

To provide an overview of the issues to be considered before application of phytoremediation and to support the implementation decision-process a flowchart for applicability of phytoremediation has been established (Trapp et al., 2014). The flowchart has been simplified from Mueller et al. (1999) for easy assessment and it is divided in two parts, flowchart A (Figure 3) and B (Figure 4). Flowchart A assesses the applicability of phytoremediation. The question being raised is whether phytoremediation at all makes sense at the site of consideration. If flowchart A successfully confirms that phytoremediation may be applicable, Flowchart B determines the applicability of the three major phytoremediation techniques, phytoextraction, rhizo- and phytodegradation and phytovolatilization.

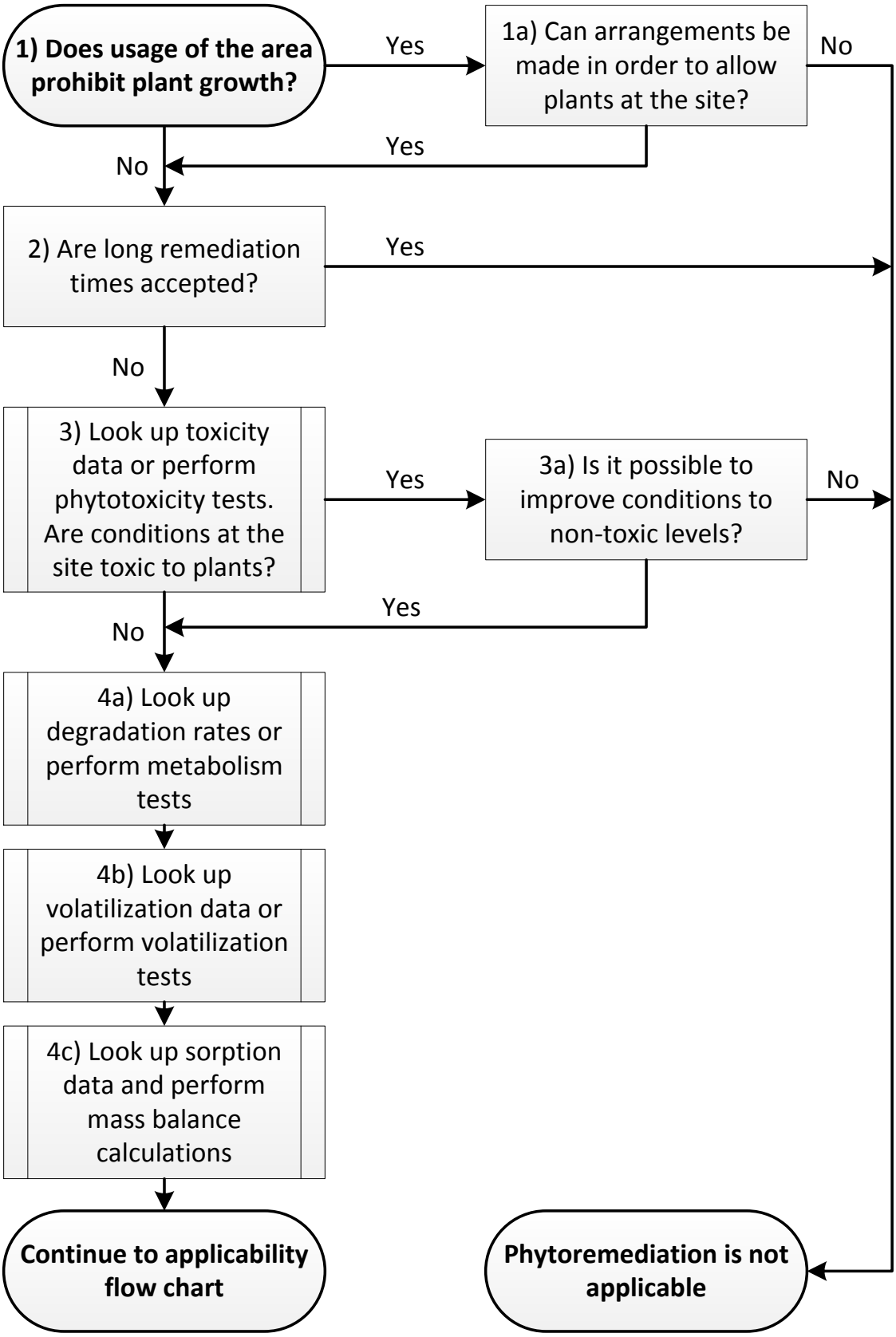

Figure 3: Flow chart A for applicability of phytoremediation (Modified from Mueller et al., 1999 as presented in Trapp et al., 2014).

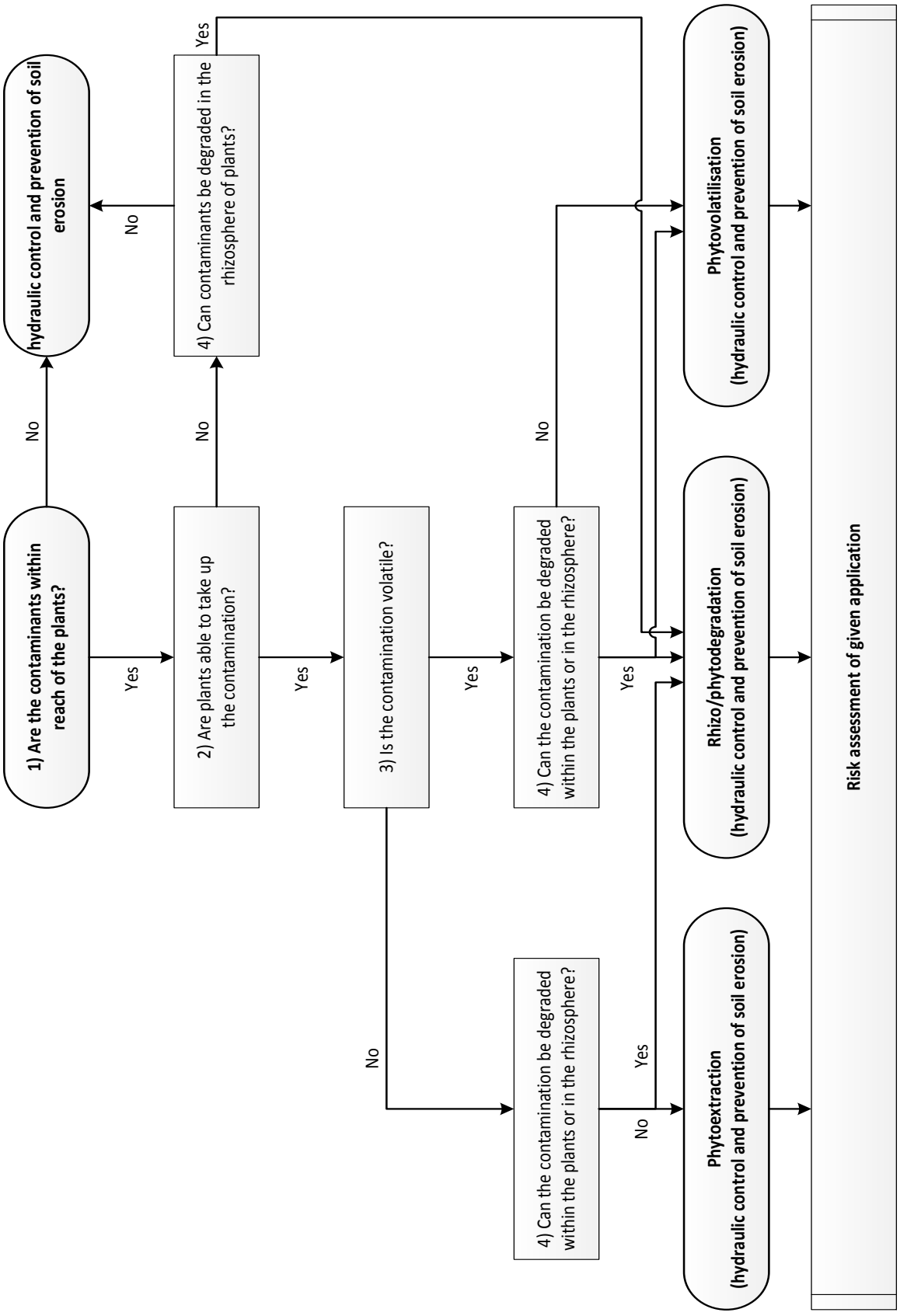

Figure 4: Flow chart B for applicability of phytoremediation techniques (Modified from Mueller et al., 1999 as presented in Trapp et al., 2014).

5.1.1 Flow chart exposition

Flow chart A and B, Figure 3 and 4, makes it possible for decision-makers to evaluate the applicability of phytoremediation and should be sufficiently self-explaining. Some of the questions raised are open-ended and requires some additional elaboration. However, Trapp et al. (2014) is referred to, for a detailed walk-through of the decision-support flow charts and for further elaboration of the individual steps.

Examining flow chart A, it is observed that the core of it consists of part 3 and 4, dealing with phytotoxicity and contaminant fate. This accentuates the need for phytotoxicity testing and fate- and mass balance modeling before the application of phytoremediation can be successfully assessed. In the following these topics are further elucidated and examples on phytotoxicity testing and fate- and mass balance studies are provided for selected compounds. Flow chart B, which builds upon the data generated or collected during flow chart A, assesses the governing processes occurring during phytoremediation and highlights the need of a risk assessment before implementation, something which is seldom reported or considered in the scientific literature, chapter 3.

5.2 Phytotoxicity testing and standardized tests

Information on phytotoxicity is required before implementation of phytoremediation, Figure 3. Phytotoxicity is not only a pivotal factor for the applicability of phytoremediation but also for the protection of sensitive plant species. In the eco-toxicity test battery implemented for chemical risk assessment within the registration, evaluation, authorisation and restriction of chemical substances (REACH) framework (EU regulation 1907/2006) and for classification, labelling and packaging (CLP) (EU regulation 1272/2008), phytotoxicity data is mandatory (European Commission, 2008). The test guidelines within the EU regulations and the predecessor guidelines are mainly based on toxicity to aquatic organisms. Phytotoxicity data has thus historically been reported according to tests following the Organisation for Economic Co-operation and Development (OECD) guidelines, International Organization for Standardization (ISO) standards or their predecessor guidelines on duckweed and green algae and not on terrestrial plants. OECD and ISO do offer guidelines on phytotoxicity tests with higher vascular plants. OECD test no. 208 is a test for seedling emergence and seedling growth (OECD, 2006a). To avoid natural extirpation of all seeds at once (e.g. droughts and fires), seeds are genetically “programmed” to germinate at various times hereby in-

troducing high uncertainties for phytotoxicity tests involving seed germination (Pallett et al., 2007). The ISO test no. 22030 is a chronic test which avoids the step of seed germination (ISO, 2005a) and is thus the best option available. In theory, chronic tests should be the basis of all risk assessments. However, they are tedious to conduct and thereby also more expensive which makes it difficult to obtain sufficient amount of data. Lastly, OECD also provides a test for vegetative vigor, test no. 227, (OECD, 2006b). The test involves spray application of toxicants and is not applicable for phytoremediation purposes of soil and groundwater pollutants that presupposes uptake thru roots. Bottom line is that no optimal acute standardized phytotoxicity test is available, why the tendency is that all laboratories evolve unique experimental setups. These unique experimental designs may be appropriate for the specific studies but it hampers cross study comparison and transparency. To illustrate how difficult it is to compare the phytotoxicity data generated, effective concentrations causing effect on 50% of the population (EC_{50}) for Cu^{2+} were collected in the scientific literature for both soil and hydroponic tests and plotted against exposure duration, Figure 5. The tests presented are not standardized, meaning that they represents several plant species, various soil and solution conditions, different light intensities etc.. It is not surprising to observe that data ranges almost 5 orders of magnitude which highlight that standardized tests or at least complete documentation of test conditions are needed if comparison between studies is desired.

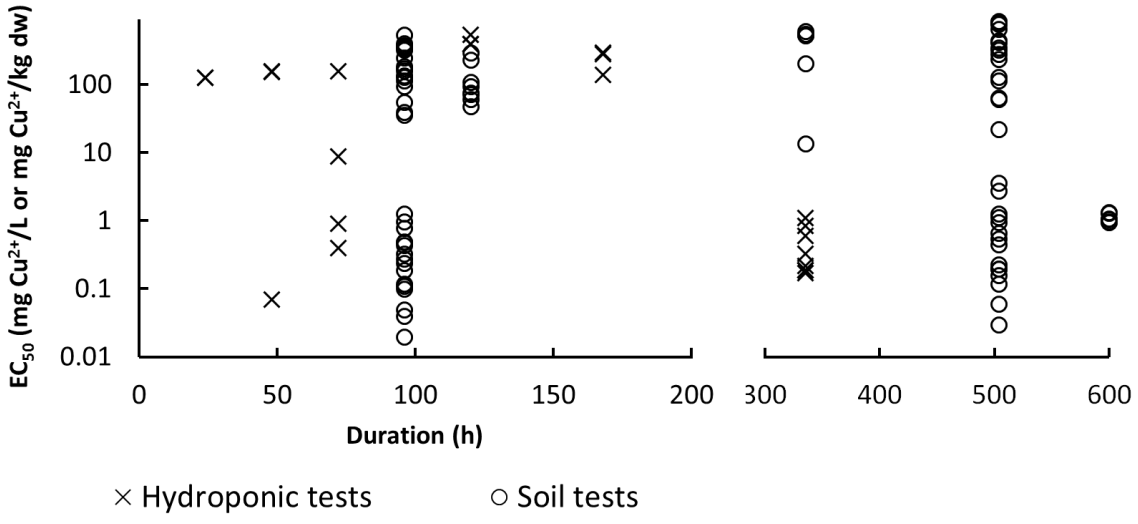

Figure 5: EC_{50}-values (mg Cu^{2+}/L or mg Cu^{2+}/kg soil dw) from the literature for various phytotoxicity studies of Cu^{2+} as a function of exposure time (h). x indicates hydroponic tests and o denotes soil tests (Clausen and Trapp, 2017 – Paper III).

5.2.1 The willow tree acute toxicity test

The willow tree acute toxicity test was first published by Trapp et al. (2000) and is available online (Trapp, 2017). The basic theory behind, is that healthy trees transpire more water than unhealthy trees. Genetically identical willow cuttings of the species *Salix viminalis* are grown in 500 mL Erlenmeyer flasks under artificial light of approximately 5000 lux at 25^{O}C and 65% humidity. The flasks are wrapped in aluminium foil to inhibit algal growth and sealed with aluminium foil (or cork stoppers) tightened with parafilm to avoid volatilization. Typically, the growth medium is 400 mL modified ISO 8692 nutrient solution which can be exchanged with contaminated soil, sand or wastewater. The toxic endpoint of the test is changes in transpiration of the individual trees compared to a group of control trees, measured by weight loss of the plant-flask-system, expressed as the Normalized Relative Transpiration (NRT%). The duration is typically 48h, 72h or 96h but can be extended up to at least 3 weeks.

$$NRT(C,t)\ (\%) = \frac{\frac{1}{n}\cdot\sum_{i=1}^{n} T_i(C,t)/T_i(C,0)}{\frac{1}{m}\cdot\sum_{j=1}^{m} T_j(0,t)/T_j(0,0)}\times 100 \qquad (5)$$

where C is the chemical concentration (mg/L), t is time (h, 0-24 h, 24-48 h etc.), T is the absolute transpiration (g/h), i is replicate 1, 2,…, n with j indicating control 1, 2,…, m (Trapp 2017).

The NRT% of control trees is always 100%. Inhibition of the treated plants by the test compound will show NRT% <100%. In addition the test can also use the growth (weight difference from start to termination) of the trees as a secondary endpoint which allows the calculation of the water use efficiency (g/L). Lastly, the test allows combined studies toxicity, uptake and metabolism.

5.2.2 Phytotoxicity

The willow tree acute toxicity test (Trapp, 2017) has been applied regularly during the past roughly one and a half decades by a handful of research groups worldwide (Clausen and Trapp, 2017 – Paper III). This makes the willow tree acute toxicity test, to the knowledge of the author, the most widely applied acute phytotoxicity test with trees not including a germination step. To date, 60 studies including 56 chemical substances has been reported in the scientific literature (Clausen and Trapp, 2017 – Paper III) and more studies

are in progress. The many studies, conducted under comparable conditions, provide a unique opportunity to compare the phytotoxicity to willows of the various substances tested. Figure 6 depicts the toxicity ranges (min. and max. observed EC_{50}-value) obtained by the willow tree acute toxicity test for 11 substances and compound groups. The most toxic chemical groups were the heavy metals and the chlorinated phenols. The chlorinated solvents (tetrachloroethylene (PCE), TCE and dichloroethylene (DCE)) and the BTEX compounds exerted low toxicity (EC_{50}-values of 40-500 mg/L) due to direct volatilization of the test compounds from solution. The least toxic substances tested were MTBE and nano-zero-valent iron particles (nZVI). nZVI showed EC_{50}-values as high as 10000 mg/L (Clausen and Trapp, 2017 – Paper III).

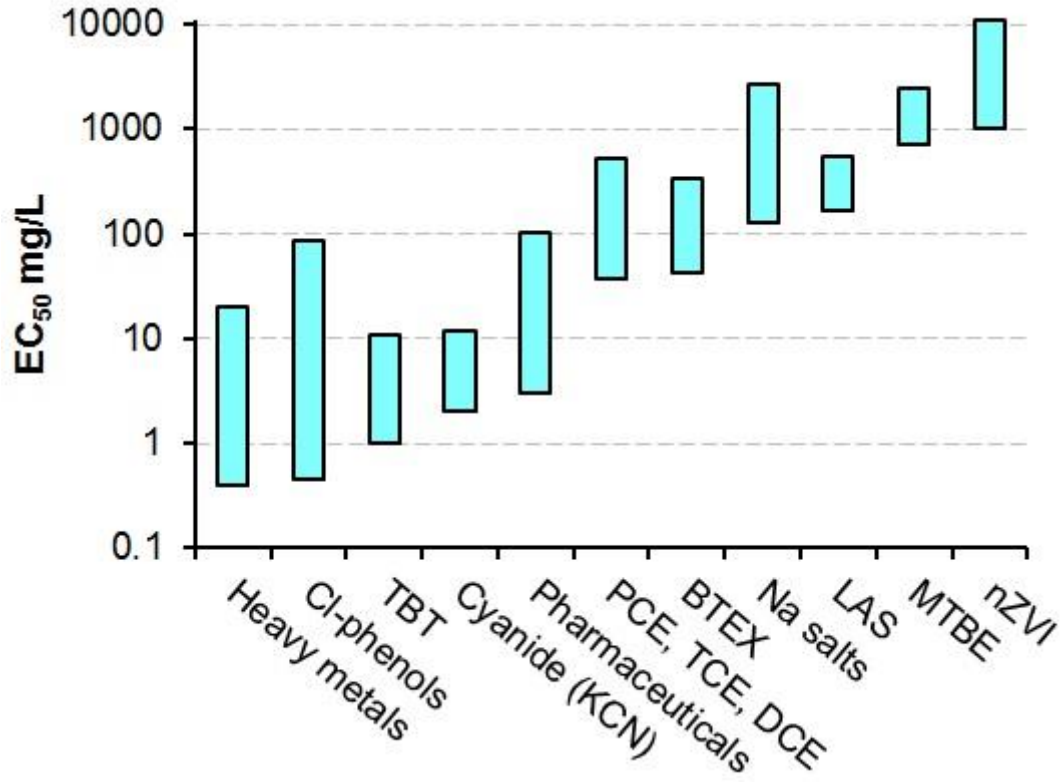

Figure 6: Ranges (min. and max.) of EC_{50}-values (mg/L) for various chemical groups observed for hydroponic willow tree acute toxicity tests (Trapp et al., 2000). Heavy metals is comprised of Cr, Cd, Cu, Se, As; TBT is tributyl tin; PCE is tetrachloroethylene, TCE is trichloroethylene, DCE is cis-dichloroethylene; BTEX is benzene, toluene, ethylbenzene, xylene; Salts is sodium chloride, sodium fluoride and potassium chloride; LAS is linear alkylbenzene sulfonate (C12); MTBE is methyl tert-butyl ether and nZVI is nano-zero-valent iron particles (Clausen and Trapp, 2017 – Paper III).

Historically, phytotoxicity data for eco-toxicological purposes has been derived by the OECD tests no. 201 or 221 (OECD, 2006c, d), the ISO tests no. 8692 and 20079 (ISO, 2005b, 2012) or their predecessor tests on freshwater

non-attached microalgae and *Lemna*. Using these algae and *Lemna* data as surrogate for phytotoxicity data on higher vascular terrestrial plants may be problematic (Fletcher, 1990).

Algae and *Lemna* are from a physiological point of view completely different from the terrestrial plants. Also, they are fully or partly submerged into the growth media allowing a high uptake of substances through diffusional processes whereas willows and most other higher terrestrial plants primarily are exposed to soil and groundwater pollutants through uptake via roots. Further, Sitte et al. (1991) stresses that single celled organisms lack important hormone systems which affect the metabolic and elimination processes in plants and Fletcher (1990) strongly advised not to use algae data as surrogate for phytotoxicity data of terrestrial plants.

To address this issue, the phytotoxicity data generated with the willow tree toxicity test for phenol and 4 chlorinated phenols were compared to phytotoxicity data of the same substances generated by the standardized OECD and ISO tests and related tests (Clausen and Trapp, 2017 – Paper III). This comparison, presented in Figure 7, shows the ranges (min. and max. observed EC_{50}-values) for phenol and the chlorinated phenols for each of the three tests. For the willow tree test 72h data was used, for the algae test 42-96h data was used and for the *Lemna* test 96-240h data was used. It is observed that all of the tests capture the increasing toxicity with increasing number of chlorine atoms attached. Further, it is observed that none of the taxonomic groups can be characterized as the most sensitive and that the three tests typically are within the range of one order of magnitude from each other. The only "outlier" is the toxicity range of the algae test with tetrachlorophenol (TeCP), which ranges more than two orders of magnitude. This broad toxicity range is most likely a result of pH and ionization and highlight the importance of reporting pH conditions when testing toxicity of weak acids and bases (Clausen and Trapp, 2017 – Paper III; Clausen et al., 2017b – Paper IV).

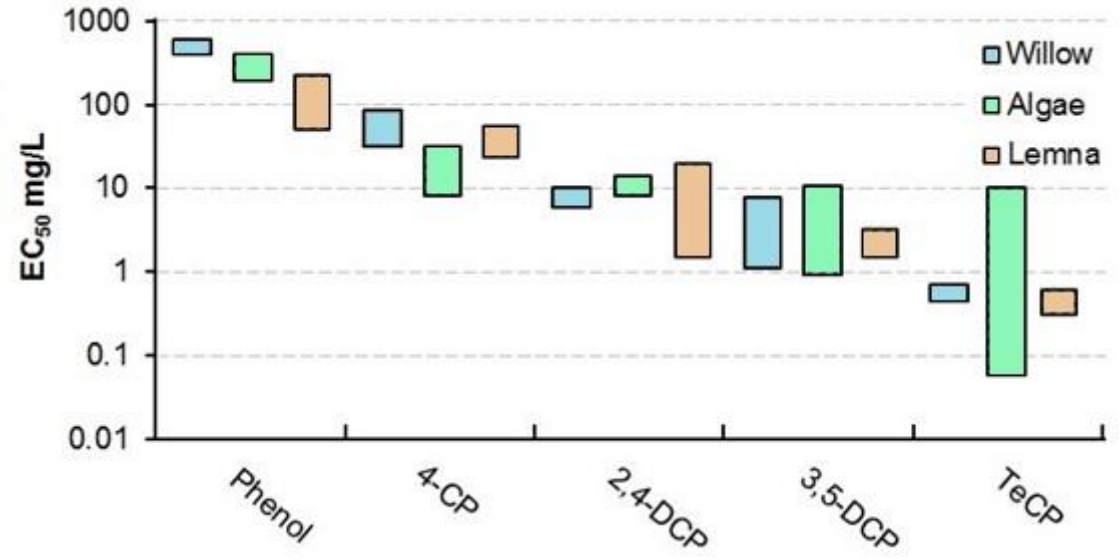

Figure 7: Observed EC_{50}-value ranges (mg/L) (min. and max.) for phenol and chlorophenols obtained with the willow tree toxicity test and standardized tests with freshwater non-attached microalgae and *Lemna*. Exposure duration is 72h for the willow tests, 48-96h for algae tests and 96-240h for the *Lemna* tests. 4-CP is 4-chlorophenol and DCP is dichlorophenol. For willows TeCP refers to 2,3,5,6-tetrachlorophenol, for algae tests TeCP refers to 2,3,5,6- and 2,3,4,6-tetrachlorophenol and for Lemna tests TeCP refers to studies of 2,3,4,6- and 2,4,5,6-tetrachlorophenol due to lack of data on 2,3,5,6-tetrachlorophenol (Clausen and Trapp, 2017 – Paper III).

5.3 Contaminant fate studies and mass balance modelling

A relevant question raised by site owners and project managers dealing with phytoremediation of soil and groundwater pollution is, how long time is it going to take? Flow chart A, Figure 3, stresses that data on contaminant fate (degradation, volatilization and uptake) has to be generated or gathered. This data form the basis for mass balance calculations which may give an initial prediction of the remediation time frame.

5.3.1 Phytodegradation and -volatilization of TCE

Laboratory experiments and field scale observations of contaminant fate are vital for the establishment of mass balance models for prediction of an approximate phytoremediation timeframe, Figure 3. With regards to fate, a very controversial compound is TCE. As described in section 3.2 some authors believe that TCE is readily taken up and degraded by enzymatic processes in plants. However, the rates of the in plant degradation are still not well described or determined.

To assess the fate of TCE in plants, the willow tree toxicity test, described in section 5.2.1, was conducted using chloride as a tracer for TCE mineralization (Clausen et al., 2017a – Paper II). The willows were grown hydroponically in chloride free nutrient solution spiked with TCE for three weeks. TCE spiked nutrient solution was added weekly to account for the solution lost by transpiration of the willows and to maintain TCE concentrations in solution above 1 mg/L for at least 2/3rd of the time. The experimental setup is demonstrated in Figure 8.

Figure 8: Photo of the experimental setup of the TCE-metabolism study in willow trees.

In an attempt to increase the degradative capabilities of the willows and their associated microorganisms, some of the willows were inoculated with specialized strains of the plant endophyte *Burkholderia cepacia* (*B. cepacia*), which holds the VM1330-pTOM-plasmid making them capable of co-metabolizing TCE (Munakata-Marr et al., 1996; Shields and Francesconi, 1996). The mutant strains used were 301C, PR1-31 and pTOM. The tests were conducted in two runs. The first run comprised two control groups, one with chloride free- and one with regular nutrient solution not exposed to TCE, Treatment 1 and 2 in chloride free nutrient solution initially exposed to 5 and 20 mg TCE/L, respectively, and a group of dead willow sticks in chloride free nutrient solution with initial exposure of 20 mg TCE/L. The second run comprised one control with no bacteria and one test group for each bacterial strain initially exposed to 25 mg TCE/L in chloride free nutrient solution.

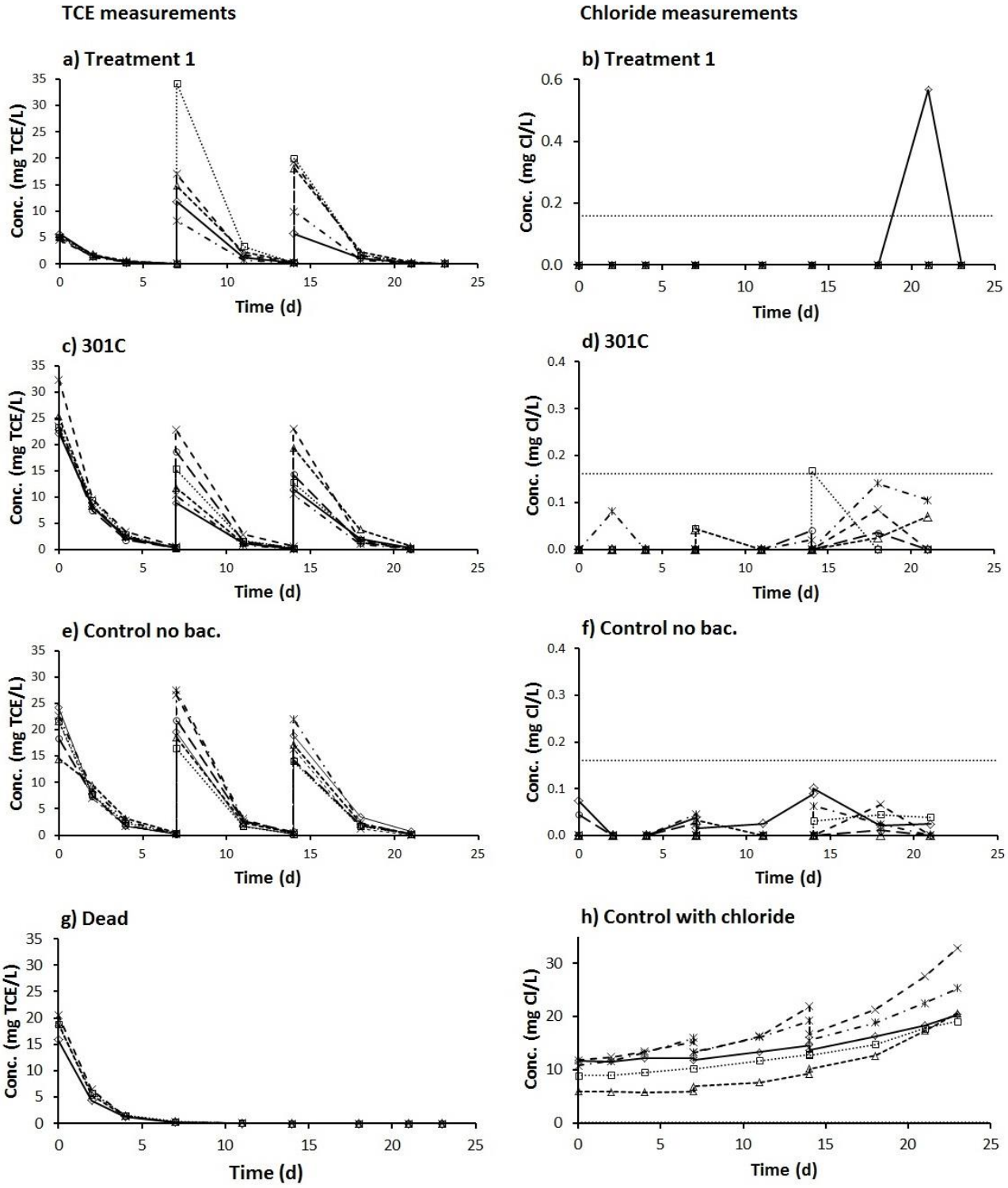

Figure 9: Selected representative measured concentrations of TCE and chloride in solution (mg/L). a,c,e and g represent TCE measurements of Treatment 1 (n=5), 301C (n=6), Control no bac. (n=6) and the dead willow sticks (n=4), respectively. b, d, f and h represent chloride measurements of Treatment 1 (n=5), 301C (n=6), Control no bac. (n=6) and Control with chloride (n=6), respectively. The dotted horizontal lines represent the LOQ for chloride in solution. (Clausen et al., 2017a – Paper II).

Selected representative measurements of TCE and chloride in solution are presented in Figure 9. The weekly addition of TCE spiked chloride free nutrient solution resulted in non-uniform TCE concentrations with the highest concentrations observed for the highest transpiring replicates as more TCE spiked nutrient solution was refilled for these replicates to uphold the water balance. In general, TCE was lost quickly from solution. Direct evaporation depleted TCE from solution within one week, Figure 9g, and accounted for approximately 96% of the total loss. Approximately 4% TCE was taken up by the willows and/or degraded.

The chloride measurements showed that little or no chloride was present in solution for all treatments but the control grown in regular nutrient solution, Figure 9b,d,f,h. Some few replicates had chloride concentrations above the limit of quantification (LOQ) but it was not consistent and in concentrations of 0.6 mg Cl/L or lower. Interestingly, the chloride concentrations in solution for the willows grown in regular growth medium increased 2-3 folds over the test duration. This phenomenon was also observed by Trapp et al. (2008) for low external concentrations of chloride and by Clausen et al. (2015) (Paper - I) for fluoride and is caused by enzymatic removal of the halogen ions from the root cells. This is further described in section 5.3.2.

To establish a mass balance, plant chloride concentrations were determined in roots, leaves and stems, Figure 10. The measurements showed that the natural differences and background levels of chloride in the plants were high. However, only the control group grown in regular nutrient solution had increased chloride concentrations in roots and leaves. The overall chloride mass balance showed no increase in chloride among the treatments, indicating that no or very little TCE had been mineralized. It was calculated that the experimental setup would capture degradation of 3% of the added TCE and degradation was thus concluded to be less. Only traces of aerobic degradation products of TCE in plants were observed (Clausen et al., 2015 – Paper II). Schöftner et al. (2016) conducted a very similar study with radionucleolabelled TCE and reach almost the same result (TCE degradation less than 1% of added TCE). From this, phytovolatilization seems to be the governing fate of TCE taken up by plants, why phytodegradation of TCE is not assessed to be applicable.

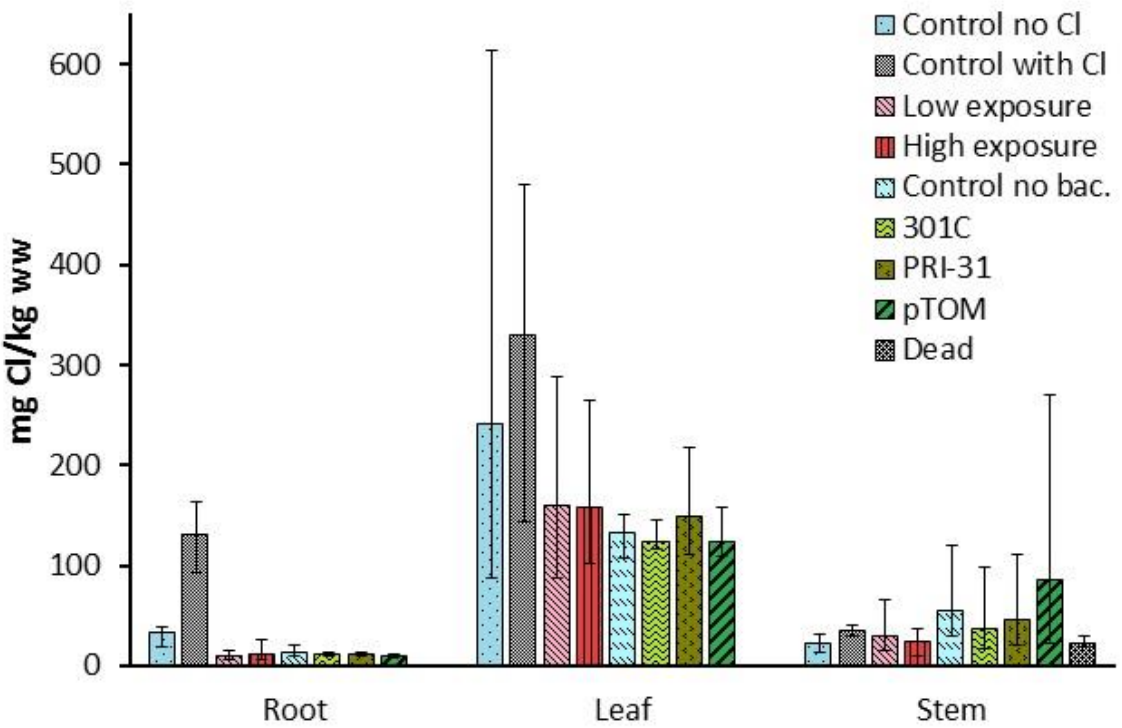

Figure 10: Mean plant tissue concentrations of chloride (mg Cl/kg fw) in roots, leaves and stems at test termination, t=23 and 21 days, for the first and second test run, respectively. Values are averages of n=5, n=4 for dead trees and n=6 for the microbial treatments. Error bars indicate the minimum and maximum observation. (Clausen et al., 2017a – Paper II).

5.3.2 Uptake and enzymatic removal of fluoride

To evaluate the feasibility of phytoremediation for a sodium fluoride contaminated site in Fredericia, Denmark, a willow tree acute toxicity test, described in section 5.2.1, was conducted, measuring toxicity of sodium fluoride and uptake of fluoride to plants, simultaneously (Clausen et al. 2015 – Paper I). Sodium fluoride serves as a good reference compound for uptake modelling of inorganic salts as it does not degrade or volatilize at test conditions (pH 7). Establishing the mass balance was accomplished by measuring fluoride concentrations in solution by a fluoride sensitive electrode. Other ions than fluoride were expected to interfere with the electrode why the test solutions were prepared in deionized water. From a toxicological point of view hydroponic experiments with deionized water is a worst case scenario.

From the measured values, the uptake of fluoride to the willows was calculated. Previously, Trapp et al. (2008) showed that chloride is pumped enzymatically out of the root cells by specific root ion channels. Supposing that these ion channels are not limited to chloride, a dynamic mass balance model with enzymatic removal following the Michaelis-Menten kinetics was applied. The model assumes passive uptake of fluoride with the transpiration stream and is

described by the differential equation:

$$\frac{dC_R}{dt} = C_w \frac{Q}{M_R} - C_R \frac{Q}{M_R K_{RW}} - \frac{v_{max} C_R}{K_M + C_R} - k_R C_R \qquad (6)$$

where dC_R/dt is the change of fluoride concentration in the roots (mg/kg), Q is the transpiration stream (L/day), C_W and C_R are the fluoride concentrations in the external solution and in roots, respectively (mg/L and mg/kg), M_R is the root mass (kg), K_{RW} is the root to xylem partitioning coefficient (L/kg), $\boldsymbol{\nu}_{max}$ is the maximum enzymatic removal rate (mg/d/kg), K_M is the half-saturation constant (mg/L) and k_R is the root growth rate (day^{-1}).

At steady-state this leads to a quadratic differential equation.

$$0 = \underbrace{\frac{K_M Q C_W}{M_R} + \frac{Q C_W C_R}{M_R}}_{\textbf{Uptake by transp.}} - \overbrace{\frac{K_M Q C_R}{M_R K_{RW}} - \frac{C_R^2 Q}{M_R K_{RW}}}^{\textbf{Translocation}} - \underbrace{K_M k_R C_R - k_R C_R^2}_{\textbf{Growth}} - \overbrace{v_{max} C_R}^{\textbf{Enzymatic removal}} \qquad (7)$$

Setting K_{RW} to 1 L/kg (no adsorption), the Michaelis-Menten parameters K_M and $\boldsymbol{\nu}_{max}$ were estimated by inverse modelling of the fluoride plant concentrations by minimizing the sum of absolute errors, Figure 11. K_M and $\boldsymbol{\nu}_{max}$ were 2 g/L and 8992 mg/kg/d, respectively. On a molar basis the $\boldsymbol{\nu}_{max}$ obtained for fluoride (0.48 mol/kg/d) was equal to the $\boldsymbol{\nu}_{max}$ obtained by Trapp et al. (2008) for chloride (0.53 mol/kg/d), indicating that the same enzyme system might be responsible for pumping out chloride and fluoride. The break-through point, the external concentration where the enzyme system collapses and the plants start to take up fluoride, was 210 mg/L. The corresponding EC_{50}-value was 129±51 (95% CI) mg F/L. A related observation is that at low external fluoride concentrations the plants are capable of pumping out the fluoride ions, thus increasing the fluoride concentrations in the external solution (Clausen et al. 2015 – Paper I). This indicates that the plants were able to detoxify fluoride at low external concentrations by use of this enzyme pump.

For the Fredericia site, the mass balance modelling shows that phytoremediation is challenged by the low plant uptake of fluoride at low external concentrations. At high external concentrations (above 200 mg/L) the plants start to

take up fluoride but are at the same time suffering from toxic effects. Phytoremediation was found not to be a feasible remediation technology at Fredericia.

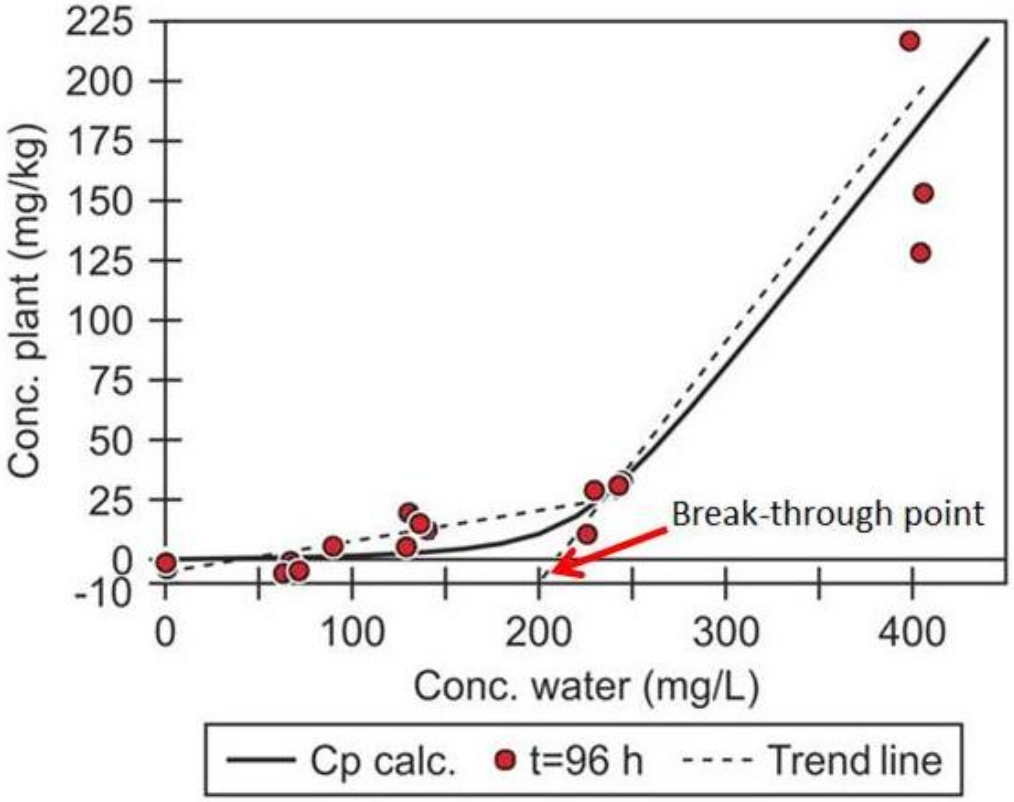

Figure 11: Fluoride concentration in willows (Conc. plant) versus fluoride concentrations in solution (Conc. water) as predicted by the non-linear mathematical model and determined experimentally (t=96h), together with two linear trend lines (Clausen et al. 2015 – Paper I).

5.3.3 Field scale mass balance studies

When estimating remediation time of phytoremediation, site specific data is required. Importantly, data on contaminant mass present at the site is needed. Examples of mass balance studies at field scale level and prediction of treatment durations are presented in Trapp et al. (2014) for a BTEX contaminated site at Szprotawa, Poland, and a trace element contaminated site at Hunedoara, Hungary. At Szprotawa an immediate reaction model was applied assuming limited BTEX degradation by limited oxygen diffusion to the subsoil. The remediation timeframe was assessed to be "*one or few decades*" for the vadose zone (Trapp et al., 2014). For the Hunedoara site a model requiring direct measurements of the bio-concentration factor (kg/kg) in plants at site was applied. The model excludes degradation and volatilization and as-

sumes passive uptake of contaminants with the transpiration stream. The remediation time at Hunedoara was assessed to be absurd long (more than 80,000 years) and clearly indicate that phytoremediation at the site is inappropriate.

6 Discussion

Much research has been conducted within the field of phytoremediation, chapter 3, and the basic concepts of the technology are well understood (McCutcheon and Schnoor, 2004). Lots of new studies are published every year trying to uncover the unknown pieces of the greater phytoremediation puzzle. Most of the published studies report amazing results showing great promises and estimates fairly short (often within a decade) remediation times for most substances (Doty et al., 2017; Gerhardt et al., 2017; Cai et al., 2016). What seems to be missing in the literature is examples of successful "full" remediation. A vast amount of the studies published report field or laboratory observations for one to three growth seasons which are then extrapolated to estimate the expected remediation timeframe. What is really needed for the phytoremediation technology to proof itself is field observations reporting the full story from initial implementation to the terminal phase where legal soil quality criteria are met, within a sufficient timeframe. Despite the long history of phytoremediation (two to three decades) there are only few to none examples of successful "full" remediation (Gerhardt et al., 2017; Chirakkara et al., 2016). This of course makes regulators, land owners and companies reluctant to implement phytoremediation.

Assessing the uptake of contaminants to plants is often done in controlled laboratory pot experiments (Chen et al., 2000; Lombi et al., 2001; Willscher et al., 2017). According to equation (*2*), the uptake of contaminants is directly correlated to the contaminated soil mass (or volume). In pot experiments the soil volume, in contrast to plant growth, is small compared to field applications. This results in severe underestimation of the remediation timeframes, even though the results obtained in the pot experiments seem promising.

A short calculation example using equation (*3*) and excluding degradation and leaching (for a trace element): With the default input values (Q = 0.8 L/d, C_S = 100 mg/kg (w.w.), K_d = 10000 L/kg, M_s = 5000 kg the change in concentration is -0.1 mg/kg (w.w.) after 1 year. Changing M_s to 20 kg (w.w.) which reflects a typical pot experiment the change in concentration is suddenly -23.4 mg/kg, more than 100 times faster than the change observed at field scale volumes. This might explain why we see many studies reporting great success at laboratory scale but only few at field scale. An example is the study by Pradhan et al. (1998) which investigated phytodegradation of PAH by three plant species in a laboratory pot experiment. They reached the conclusion that that phytoremediation of PAH is effective even though the soil

volume is less than 1L. This explains why phytoremediation was highly praised during the early stages when it evolved and how high expectations were transformed into mistrust and reluctance - A shadow which is still partly surrounding the technology. Anyway, phytoremediation may take longer than first expected from the initial pot experiments.

6.1 Knowledge gaps

As described in chapter 4, Equation (*2*) presents a generic mass balance of phytoremediation. It also points out where our knowledge is insufficient, namely the lumped removal rate k_L. When the contaminants are readily degraded and not volatile the assumption that $k_L \approx k_{deg}$ is fairly good. If the microbial degradation is high, the compounds are less likely to be an environmental problem in the first place. However, if microbial degradation is slow, the importance of other processes is much more pronounced making the assumption less accurate. Degradation rates for many organic compounds are available in the literature (Birch et al., 2017; Juhasz and Naidu, 2000 and more) but such rates should always be adjusted to reflect the site specific field conditions. The fact that field sites are much more complex than controlled laboratory studies is a challenge making field observations pivotal for the final assessment of feasibility.

Contaminant fate in plants is not only important from a phytoremediation perspective but is relevant for both pesticide designing and risk assessment purposes too (Dumas et al., 2017; European Commission, 2003). Many of the plant processes involved are not well understood or known, chapter 5, why studies like Clausen at al. (2017a) (Paper II), for TCE, and Clausen et al. (2015) (Paper I), for fluoride, are essential. The fact that some plants have an outwards directed enzymatic pump for removal of specific ions have a major impact on the plant uptake and thus also on the effect of phytoremediation. We know that the enzymatic removal can pump out both chloride and fluoride from the plant roots (Clausen et al., 2015 – Paper I and Trapp et al., 2008) but this might be true for several other substances. Further, other processes might influence uptake and fate of contaminants in plants. For compounds such as sodium fluoride and sodium chloride phytoremediation seems to be of limited use as the plants either avoid the contaminants by pumping them out or wilt and die if their enzymatic systems are overloaded.

A range of both dynamic and empirical models have been established to estimate the uptake to and fate of compounds within plants (Trapp, 2004; Gobas et al., 2015). The models available seem to perform well for most neutral

organic compounds but predictions for ionized compounds are weak (Trapp, 2004; Gobas et al., 2016). In general, there is a high demand for high-quality standardized fate studies of contaminants in plants, in order to derive sufficient input data for the prediction models (Fantke and Juraske, 2013). Finally, the models developed build upon measured laboratory derived exposure concentrations or empirical and mechanistic modelling. The existing models greatly lack large high-quality field datasets for calibration and validation purposes (Gobas et al., 2016).

6.2 Feasibility and limitations

The main limitations of phytoremediation are phytotoxicity (Clausen and Trapp, 2017 – Paper III), the often long remediation times (Clausen et al., 2015; Trapp et al., 2014) and plant root depth (Pilon-Smiths, 2005). These three issues alone make phytoremediation unattractive or unfeasible in many cases.

Assessing the feasibility of phytoremediation for specific compounds, or mix of substances, at a specific site is not always an easy task. To ease the assessment task Figure 3 and 4 offer an easy step by step check list for the applicability of phytoremediation. Further, this thesis identified some good rules of thumb for an initial assessment. In general, phytoremediation or especially microbial enhanced phytoremediation is worth considering for contaminants which are easily degraded aerobically, where degradation is limited by oxygen supply or for compounds with low K_d. Of course the prerequisites, described in chapter 2, still have to be met. Good examples of such compounds are BTEX, PHC and nutrients. The chlorinated solvents, with TCE as a reference substance, are only degraded in a very limited amount in the plants. However, this is controversial. Newman et al. (1999) found almost complete degradation of TCE in plants while experimental work conducted in this thesis (Clausen et al., 2017a - Paper II) and Schöftner et al. (2016) found less than 3% and 1% mineralization, respectively. The major TCE loss was estimated to be direct volatilization and phytovolatilization. The degradation observed by Newman and co-authors might be due to specific plant endophyte activity as observed by Doty et al. (2017).

Compounds such as trace elements and radionuclides have high K_d-values and are not degraded, Table 3. From a first glance these compounds are not suited for phytoremediation due to their strong sorption to the soil matrix. Great focus has been given to enhance phytoremediation of these compounds either by use of hyperaccumulating plants or by use of chelating agents

(Chehregani et al., 2009; Sarwar et al., 2017). Addition of chelates increases the mobility and thus the availability of many trace elements and radionuclides. However, the plant uptake is not correspondingly increased (Evangelou et al., 2007), leading to high amounts of trace elements and radionuclides available for leaching, run off, other transportation processes and exposure to humans and wild life. Chelating agents should be used with care as they hold the potential to negatively affect the risk to the environment and human health. Hyperaccumulators may be a good solution for some sites. The accumulating species are highly specialized and often only thrive at special conditions, why specific plants are needed for specific regions. There is also a risk of releasing potential invasive species. Further, the extraction by hyperaccumulating plants is typical limited by slow growth and transpiration (Cunningham and Ow, 1996).

Engineered plants do hold a great potential to solve the issue of limited extraction rates but the scepticism towards genetically modified organisms (GMO's) do constitute a barrier in the western world (Lee, 2009; Linacre et al., 2003). GMO's are controversial and should not be released to the environment without thorough ethical considerations. Another important issue to consider is environmental and human health risks. The fact that contaminants accumulate in the plant tissue may introduce a threat to wildlife grazing on, or humans harvesting and eating, the plants.

For degradable organic compounds microbially assisted phytoremediation seems to have a lot of potential, if appropriate microorganisms can be identified and successfully inoculated. Recently, focus has been on screening and identification of indigenous microbes to find special microbial features useful in phytoremediation (Paredes-Páliz et al., 2016; Syranidou et al., 2017; Tank and Saraf, 2010). The microbes may enhance rhizospheric or in plant degradation, increase the availability of the contamination to the plants or increase the resistance and growth of the plants (Feng et al., 2017; Glick, 2010; Mesa et al., 2017; Rajkumar et al., 2012). Lists of appropriate microorganisms for various compounds and purposes and possible host plants are available (Feng et al., 2017; Thijs et al., 2017) and new interesting studies are frequently released. Equation (*2*) shows that microbial degradation of contaminants in the rhizosphere is far more important than the removal by plant uptake if degradation is occurring. Therefore, microbially assisted phytoremediation seems to be the most promising phyto-application. There is also the possibility of designing specific degrader bacteria fit for plant inoculation, section 3.2.

However, this takes the discussion back to the ethical dilemma of GMO's and their release to the environment.

Phytoremediation is no rapid technology, with remediation times often longer than a decade – sometimes even several decades or centuries (Clausen et al., 2015 – Paper I; Trapp et al., 2014). These very long remediation timeframes put a natural boundary to where phytoremediation is feasible. Site owners typically desire fast remediation in order to reuse or redevelop the site (Song et al., 2018). Unless "green areas" are part of the future development strategy, phytoremediation will have difficulties competing against other, faster, and already established remediation technologies. The land value plays a major role for the remediation of a site. Urban brownfields (unused or underused contaminated or perceived contaminated sites) are typically much faster regenerated than rural sites, solely due to the economic incentive (Trapp et al., 2014).

One of the major benefits about phytoremediation is that the implementation costs are low – varying price dependent of plant species and site location. As an example the implementation of phytoremediation with willows in Denmark would cost approximately 2 DKK (~0.3 USD) per willow cutting plus the labour wage. A rough estimation of the implementation cost is 100,000 DKK (~15,500 USD) for a site of 1 ha assuming one tree per m^2, 10 monitoring wells of 5,000 DKK (~784 USD) per well and 30,000 DKK (~4,700 USD) for wage and equipment. Additional costs may be needed for pretreatment. The total cost of phytoremediation is strongly dependent on the remediation time, which again depends on the contaminants and site characteristics. If one sample costs 500 DKK (~78 USD) and monitoring of the site is done once per year in 10 years, the total costs would be 600,000 DKK (~94,000 USD). For a 20 year remediation the costs would be 1,100,000 DKK (~170,000 USD). In comparison, excavation of such an area would cost approximately 100 million DKK (~15.7 million USD) assuming that 2m top soil is removed, with a density of 1.6 t/m^3 and a deposition cost of 300 DKK (~47 USD) per ton contaminated soil.

For application of phytoextraction, there might be costs involved in seasonal seeding and harvesting. In some cases phytoremediation may even be regarded as resource production, e.g. timber, biomass for energy production, feedstuff for animals or food. An example of the latter from California, USA, is the production of selenium-enriched cactus fruits at selenium and salt contaminated soils (Bañuelos et al., 2011; Schiavon and Pilon-Smits, 2017).

Phytoremediation is thus especially well-suited for large rural areas with low land value and with shallow disperse contamination. Alternatively, phytoremediation can be implemented as the last polishing step after the application of other technologies (Schnoor et al., 1995), e.g. excavation of the phytotoxic hot spot area with subsequent phytoremediation.

Due to the low implementation cost, phytoremediation will in many cases be a better alternative than monitored natural attenuation for most organic compounds, as the plants will enhance the contaminant removal (Doty et al., 2017; McCutcheon and Schnoor, 2004). Besides, plants provide aesthetic beauty to sites which are unplanted and prevent dust and washout from the area.

6.3 Standardized procedures and testing

It is evident that the phytoremediation technology is limited by phytotoxicity, chapter 5. Observing Figure 5 gives a good indication of how important the standardized tests are, if comparison between toxicity tests is desired. At least, test reporting requirements should be standardized for complete documentation, why the phytotoxicity tests performed today with terrestrial plants are highly incomparable, chapter 5. The testing of chemicals toxicity to aquatic organisms has been standardized since 1981 for eco-toxicity purposes (OECD, 2017). Protocols for testing terrestrial organisms are much less developed (Gobas et al., 2016).

The presented overview of collected phytotoxicity data generated by the willow tree acute toxicity test, Trapp et al. (2000), for 11 substance groups including 56 substances provides much needed insight in at which concentration levels phytoremediation may be feasible, Figure 6. The data is generated by the same test-protocol and are thus comparable; however, a much more comprehensive database is needed to support the application of phytoremediation. Therefore a phytotoxicity database consisting solely of standardized (comparable) data is recommended. Such a database should also include various plant species for easy comparison of plants. Before this is obtainable, new standardized acute phytotoxicity tests with higher vascular terrestrial plants, avoiding the step of seed germination, need to be established and available.

A candidate for such a test is the acute willow tree toxicity test by Trapp et al. (2000). The test holds several advantages. The test is easy to conduct, robust, fast (results available after 48-96h) and inexpensive (costs of 30 willow

sticklings, chemicals, balance, glassware and wage) and requires no advanced equipment (a balance and a suited place to grow the willows – a greenhouse or a fume hood). For most substances it is easy to establish a mass balance of the compounds thus making it possible to study the relationship between uptake and effects (Clausen and Trapp, 2017 – Paper III). These types of data are needed when performing plant uptake models and provides pivotal information for assessing the feasibility of phytoremediation (Gobas et al., 2016). The test also makes it possible to change test parameters one by one, observing the effects of e.g. temperature, CO_2-level, pH and more. For weak acids and bases, such as the chlorinated phenols and many pharmaceuticals, the pH is pivotal for the toxicity of the compounds (Clausen and Trapp, 2017 – Paper III; Clausen et al. 2017b – Paper IV). The main shortcoming of the test is the high volume of test solution needed (400 mL per cutting), which generates a substantial amount of chemical waste and makes the required amount of test chemical high. However, the amount of test solution needed is difficult to reduce due to the water volumes needed for sustaining the transpiration of the willows (Clausen and Trapp, 2017 – Paper III). Further, the test concentrations in solution may not stay constant. E.g. if water is taken up faster than the test compound (Clausen et al., 2015 – Paper I), if the test compound is degraded in solution or lost by volatilization (Clausen et al., 2017a – Paper II). The toxicity of volatile substances will be underestimated by the test due to direct volatilization from solution. It is not possible to do a fully hermetic test system when testing living trees, although the direct volatilization can be markedly minimized by easy means, e.g. tightening the aluminium foil with parafilm and/or by addition of floating plastic balls to the test flasks. Willows contain high amounts of salicylic acid (active substance in many pain killers) which reduces the plant stress (Wani et al., 2017). This makes willows less sensitive in toxicity tests but makes them better suited for phytoremediation purposes.

One of the highlighted issues to be considered before implementation of phytoremediation is the risk assessment, Figure 4. Risk assessments for phytoremediation sites are seldom reported in the literature (Linacre et al., 2003). Many organic compounds, e.g. TCE, are either degraded in the plants, in the rhizosphere or released to the atmosphere where they are subject to rapid photolysis (Mohseni, 2005). Releases to the atmosphere happen with the transpiration why it occurs at daytime where radiation, and thus the photodegradative capacity is high. A risk assessment should also consider intermediate degradation products as these might be problematic.

Phytoextraction of toxic substances may be more problematic. The plants are used to extract and up-concentrate the substances why there might be risks for wildlife grazing in the area or humans utilizing the crops. Some heavy metals like mercury might even be transpired to the atmosphere (Heaton et al., 1998). Further, with phytoextraction contaminated biomaterial is most likely produced in high quantities. Before the implementation, there must be a plan for how to handle/expose of the contaminated material. These types of considerations should be integrated in a common phytoremediation risk assessment protocol. Risk assessment should therefore be mandatory for all applications of phytoremediation.

7 Conclusions and outlook

The aim of this thesis was to assess the feasibility of phytoremediation for common soil and groundwater pollution. This was accomplished by scrutinizing the literature for relevant data and experiences with plants as a remediation technology and by conducting relevant experimental work with selected compounds. Subsequently, several knowledge gaps and obstacles for the phytoremediation technology were identified and discussed.

A generic mass balance was established for a conceptual phytoremediation setup. The mass balance revealed that the governing loss process of contaminants is degradation in the rhizosphere for most organic compounds and not plant uptake. For non-degradable substances the governing loss of contaminant mass is by plant uptake, which is controlled by the transpiration rate and the soil/water distribution coefficient. In general, phytoremediation takes much longer time than predicted by many experimental studies as they do not take into account the differences in soil volume between pot and field experiments. The remediation time, or the extraction efficiency, of phytoremediation is assessed to be the main obstacle of the technology.

The work done within this thesis highlights that there are unknown plant processes that influence uptake and fate of contaminants in plants. These processes may be of marked importance for the success of phytoremediation of influenced compounds.

Phytoremediation is limited by phytotoxicity why phytotoxicity data on higher terrestrial plants is pivotal. The lack of standardized tests and reporting requirements has led to many incomparable studies thus hampering the transparency of the field. The willow tree acute toxicity test by Trapp et al. (2000) was proposed as a candidate for a standardized test and an overview of the data generated by the guideline was presented. Establishment of a phytotoxicity database consisting solely of standardized test results (comparable data) on higher terrestrial plants, not including seed germination, would benefit the feasibility of phytoremediation.

Currently, there is no official guidance document available on how to implement phytoremediation. This thesis proposes a simple flow chart for assessment of the feasibility and applicability of phytoremediation, meant to ease the implementation decision-process of stakeholders considering phytoremediation.

Phytoremediation is not as fast and effective as claimed by many authors; however, there are windows of opportunities. Phytoremediation is assessed to be feasible for nutrients and organic pollutants which can be degraded aerobically. Most trace elements are too strongly bound to the soil matrix to be available for plant extraction and are thus not feasible for phytoremediation. There might be means to address these issues, e.g. hyperaccumulating plants, soil amendments and engineered plants but these techniques are not fully developed and might lead to exacerbation of the environmental conditions.

Lastly, phytoremediation has to be proven by providing examples of field-scale applications where the soil quality criteria are reached within acceptable timeframes.

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9 Papers

I **Clausen LPW**, Karlson UG, Trapp S (2015). Phytotoxicity of sodium fluoride and uptake of fluoride in willow trees, *International journal of phytoremediation*, 17(4), 369-376.

II **Clausen LPW**, Broholm MM, Karlson UG, Trapp S (2017). Test of aerobic TCE degradation by willows (*Salix viminalis*) and willows inoculated with TCE-cometabolizing strains of *Burkholderia cepacia*, *Environmental Science and Pollution Research*, DOI 10.1007/s11356-017-9420-8.

III **Clausen LPW**, Trapp S (2017). Toxicity of 56 substances to trees, *Environmental Science and Pollution Research*, DOI 10.1007/s11356-017-9398-2.

IV **Clausen LPW**, Jensen CK, Trapp S (2017). pH dependent toxicity of 2,3,5,6-Tetrachlorophenol to willows. *Human and Ecological Risk Assessment: An International Journal*. Short communication. *Submitted*.

In this online version of the thesis, **paper I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from.

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